The Thesis Committee for Stephanie Denise Smith
Certifies that this is the approved version of the following thesis:

Tracing organic matter pathways in marine food webs using fatty acids and compound specific stable isotope analysis

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

James W. McClelland, Supervisor

Kenneth H. Dunton

Benjamin D. Walther

Tracing organic matter pathways in marine food webs using fatty acids and compound specific stable isotope analysis

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Stephanie Denise Smith, B.S.

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Dedication

I dedicate this thesis to my parents Stiles and Melanie, my stepparents Matt and Kathy, and my siblings Caitlin and Steven, for their endless support and encouragement to pursue my childhood dreams of becoming a marine biologist. To my uncle Steve, who inspired me as a little girl and gave me hope that I would "be something great, someday". To my cousin Scott, who is like a brother to me, who always believed in me, and helped me to get my Aggie Ring (Gig 'Em & Hook 'Em!). I also want to dedicate this to two fabulous women scientists who mentored me throughout my undergraduate and graduate research, Sally Walker and Tara Connelly. Without their patience, guidance, and willingness to help, my achievements would not have been possible. And finally, to my colleague, neighbor, friend, and partner, John Mohan, for challenging me to become a better scientist, and for helping me to take it easy and enjoy the simple things in life.

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Abstract

Tracing organic matter pathways in marine food webs using fatty acids and compound specific stable isotope analysis

Stephanie Denise Smith, M.S.MarineSci.

The University of Texas at Austin, 2015

Supervisor: James W. McClelland

Organic matter inputs to the marine environment vary over seasonal and spatial scales, altering the type and availability of food sources for marine consumers. It is important to identify diet in order to understand basic ecology, characterize trophic interactions, and predict consequences of biotic and abiotic change within a community. Methods of direct observation of diet and feeding can be difficult, so indirect methods have been developed such as analysis of gut contents and fecal pellets. However, these methods only represent a snapshot of the last meal, and provide information about what was ingested, but not what was actually incorporated into consumer tissues. Therefore, biogeochemical approaches such as fatty acid (FA) and stable isotope analyses have been developed, which provide a time-integrated measure of diet. Further, stable isotope measurements of specific FA markers can be used to identify carbon sources, and can be applied to a variety of food web studies (Iverson et al., 2004). The purpose of this research is to examine the linkages between organic carbon sources and trophic transfer by consumers. To achieve this, we use FA biomarkers and compound specific stable isotope

analysis (CSIA) to trace carbon cycling. This study has two main components: environmental sampling and experimental research. Chapter 1 demonstrates the use of these tools for elucidating seasonal trophic linkages in invertebrates collected from the Alaskan Arctic coast. Overall, invertebrate diets were characterized by terrestrial, detrital, and carnivorous sources in winter and spring, with a shift toward autochthonous diatom-based diets in summer. Our results demonstrate the importance of terrestrial organic carbon as a subsistence food source in winter, whereas *in situ* production in summer was critical for accumulating FA stores rich in essential FAs. Chapter 2 is an experimental feeding study designed to quantify the incorporation rates of 18:2n-6 from diet to tissue in Atlantic croaker. Liver tissues accumulated FAs more quickly than muscle tissues, but both tissues reached equilibrium at 5 to 7 weeks. From these experiments, quantitative assessments of diet sources can be made with confidence when using FAs to understand trophic interactions of Atlantic croaker and other similar species.

Table of Contents

List of Tables

List of Figures

Introduction

Understanding trophic ecology of individuals, populations, and communities is necessary for predicting ecological consequences of biotic and abiotic change. A variety of direct methods can be used to characterize diets and trophic interactions including: observation of feeding, gut content and fecal content analysis. Yet, these direct methods may overestimate the importance of recently consumed food sources, and underestimate soft and easily digestible foods (Kelly and Scheibling, 2012). It is also impractical for elucidating diets of invertebrates that have a wide range of feeding modes and dietary items. Therefore, indirect methods such as stable isotope and fatty acid analysis have been developed to provide a time-integrated measure of diet over longer time scales. The goal of this research is to elucidate trophic relationships via transfer of fatty acids and compound-specific stable isotopes in marine systems.

FATTY ACID BIOMARKERS

Lipids are high-energy biomolecules used for energy storage and as structural components of cell membranes of all living organisms. They can be extracted using chloroform and methanol (following Folch et al. 1957), and further separated from non-lipid compounds into 16 different lipid subclasses. These 16 lipid subclasses provide a wealth of information about origin (e.g. biogenic or anthropogenic), caloric content, age of the material, condition of degradation, and nutrient limitation of algae (Parrish et al., 2000). Of these subclasses, fatty acids (FAs) are one of the most important lipid biomarkers for trophic studies, as they represent the "building blocks" of many other lipids (Iverson, 2009). FAs are generally not degraded and remain intact within tissues (Iverson, 2009). Also, unlike other dietary nutrients, lipids are stored in body tissues and can accumulate

over time, therefore representing dietary intake over longer time scales. Fatty acids have a variety of structures and because certain FAs can be transferred to consumers unaltered, they can be used as a biomarker for carbon sources (i.e. the producer). By tracing the flux of a specific FA between the tissues of prey and predators, marine food web linkages can be constructed (e.g. Budge et al., 2008; Iverson 2009).

The accepted nomenclature for FA naming is in the form of "*A*:*B*n-*X*", where *A* represents the number of carbon atoms, *B* the number of double bonds, and *X* the position of the double bond closest to the terminal methyl group (Parrish et al., 2000). In general, marine primary producers synthesize FAs ranging from 14 to 24 carbon atoms. Algae are typically the only organisms that can produce the long-chain polyunsaturated FA (PUFA) 20:5n-3 and 22:6n-3 *de novo*. These PUFA can be found in higher trophic level organisms, indicating trophic transfer from the prey source (Table 0.1). Specific FAs can also be used to differentiate between diatoms and dinoflagellates (e.g. ratios of 16:1/16:0), terrestrial plant biomarkers (e.g. 18:2n-6 and 18:3n-3), and bacterial biomarkers (Table 0.1).

Aside from being indicators of source-specific carbon, FAs also serve a nutritional role for marine consumers. It is widely accepted that LIN (18:2n-6), ALA (18:3n-3), ARA (20:4n-6), EPA (20:5n-3), DPA (22:5n-3) and DHA (22:6n-3) are the most important long-chain PUFAs in some invertebrates, mammals and fishes, because they are required for normal somatic growth, neural development, reproduction, eicosanoid production, survival, and pigmentation (Parrish, 2009; Sargent et al., 1999). These specific PUFAs are known as the essential fatty acids (EFAs), because they cannot be synthesized *de novo* and must be supplied by the diet (Bell and Wolfgang, 2010; Tocher, 2003). The EFA requirements vary among species and are influenced by a range of factors, but it is a general rule that larval and juvenile fishes tend to have a higher requirement for n-3 long-chain

PUFA than in later life stages. For marine fish species, like red drum (*Sciaenops occeltus*), the requirements for n-3 long-chain PUFA are in the range of 0.4%-3.7% of dry diet (Bell and Wolfgang, 2010). Therefore, identifying the trophic transfer of EFA in invertebrates and juvenile fishes is important for estimating the overall health and production within a community (Müller-Navarra et al., 2004).

Qualitative analysis of FA profiles can be used to explain spatial and temporal variation in diets of consumers by using a suite of multivariate statistics. This approach relies on recognizing differences in certain FAs or ratios among FAs that can only be attributed to one or a few prey types, indicating their prevalence in the diet (Iverson 2009). More recently, FAs have been used to develop a quantitative assessment of predator diets known as quantitative fatty acid signature analysis (QFASA) (e.g. Iverson et al., 2004). This statistical technique is based on the assumption that marine fishes and invertebrates have distinct FA signatures, and that these signatures are accumulated in predator tissues in a predicable way. By comparing the FA composition of all potential food sources to that of the predator, an estimate of diet can be obtained (Budge et al., 2006). This first step of QFASA is to calculate weighting factors, known as calibration coefficients. These calibration coefficients are used to determine the extent of FA metabolism or deposition into tissues (Iverson et al., 2004).

While FA analysis alone can provide an abundance of information, there are certain limitations with this method that can impede identification of the source signature. For example, some organisms can synthesize FAs *de novo* or modify FAs from other FA precursors (e.g. elongation, saturation), inhibiting the reliability of the source trophic markers (Bec et al., 2011). To overcome these biases, compound specific isotope analysis

(CSIA) of individual FAs can be used to relate individual FAs to their respective carbon source.

COMPOUND SPECIFIC ISOTOPE ANALYSIS OF FATTY ACIDS

Most trophic studies that take advantage of stable isotopes utilize bulk measurements of carbon, nitrogen, and to a lesser degree, sulfur to make predictions about diet and trophic level enrichment. Bulk stable isotope analysis (BSIA) of total organic carbon is used to determine broadly defined source contributions (i.e. terrestrial vs. marine carbon), but greater specificity is not possible. New developments in CSIA are thought to amend the limitations of BSIA by targeting compounds that are characteristic of specific sources. Two advantages of CSIA over BSIA is firstly, this technique avoids the problem of isotopic routing, and secondly, $\delta^{13}C$ values for individual FAs can be collected simultaneously, thereby increasing the accuracy of diet assessments (Budge et al., 2011). While CSIA can be more costly and time consuming, combining it with BSIA could lead to a more holistic understanding of stable isotope transfer.

Budge et al., (2008) demonstrated the successful application of fatty acid stable isotope (FA-SI) analysis in an Arctic marine food web. In this study, CSIA was used in combination with FA analysis to determine the relative contribution of ice algae and pelagic phytoplankton to the overall productivity off the coast of Barrow, Alaska. Samples from each trophic level (i.e. diatoms to bowhead whales) were collected and analyzed for the specific FAs only synthesized by diatoms. Because ice algae and pelagic phytoplankton have unique $\delta^{13}C$ values for the specific FA 16:4n-1 and 20:5n-3 (EPA), a two end member mixing model could be used. The resulting mass balance equation showed that ice algae contributed between 24-71% of the overall productivity of the system, and a reduction in productivity could be expected with future sea-ice loss. This study demonstrated that CSIA

is a valuable tool for tracing carbon flow throughout food webs, and can be applied to related studies.

CSIA of FAs is expected to amend the limitations of traditional methods like BSIA and FA analysis alone. Yet, in order for CSIA of FAs to be reliable, the individual FAs must accurately reflect the $\delta^{13}C$ value of the consumer's diet in a predictable way. While there have been studies demonstrating the usefulness of CSIA of EFAs for food web studies, few have addressed the issue of isotopic fractionation. Bec et al. (2011) and Budge et al. (2011) tested the extent of fractionation using controlled feeding experiments. In both cases, the authors found that the carbon stable isotope values of some fatty acids (e.g. EFA) in consumers were closely related to diet, whereas other non-essential FA were substantially altered via metabolic processes within the consumer. But there are only a few studies beyond these two that apply FA-SI analysis to trophic transfer studies (e.g. Gladyshev et al., 2012; Graham et al., 2014). Therefore, there is a great need for more controlled diet studies to explain the patterns of isotope fractionation and advance the field of CSIA forward.

Table 0.1. Sources and characteristics of FA biomarkers used in marine trophic studies, as reviewed by Dalsgaard et al. (2003) unless otherwise noted.

FA biomarkers	Character or source	References
Polyunsaturated FA (PUFA)	labile; high nutritional quality	
Saturated FA (SFA)	refractory; low nutritional quality	
	detritus (particularly 18:0)	Mayzaud et al. (2013)
n-3 PUFA	autotrophs; high nutritional quality	
n-3/n-6	high nutritional quality	
∑16:1/16:0	Diatom (>1)	Claustre et al. 1988
20:5n-3 + 22:5n-3 + 22:6n-3	Marine autotrophs	
18:2n-6 + 18:3n-3	Terrestrial plants when >2.5% of total FA	Budge and Parrish (1998)
	Green algae; macroalgae	Graeve et al. (2002)
∑20:1 + ∑22:1	Copepod	Falk-Petersen et al. (1987)
22:0 + 24:0	Terrestrial plants	
∑odd-numbered + ∑branched-chain FA	Bacteria	Stevens et al. (2004)
18:1n-9	Animal detritus; animal tissue; carnivorous feeding	Graeve et al. (1997)
DHA/EPA (22:6n-3/20:5n-3)	Dinoflagellate (>1) versus diatom nutritional index	Budge and Parrish (1998)
EPA/ARA (20:5n-3/20:4n-6)	Eicosanoid activity	Sargent et al., 1999

Chapter 1: Understanding seasonal trophic linkages via fatty acid profiles, and bulk, lipid and fatty acid-specific stable isotopes in Arctic marine invertebrates

ABSTRACT

Climate change is having profound impacts on Arctic ecosystems with important implications for coastal productivity and food web dynamics. The goal of this research was to determine seasonal variations in diets of 16 key invertebrate taxa in lagoon ecosystems along the Alaska Beaufort Sea coast. We used a combination of fatty acid biomarkers and stable carbon isotope measurements of whole animals, total lipid extracts, and individual fatty acids to identify the relative importance of terrestrial organic matter and in-situ primary production to their diets. Invertebrates collected during full-ice cover (April), ice break-up (June) and open water (August) displayed shifts in seasonal contributions of terrestrial and marine food sources. An amphipod collected in April had higher proportions of terrestrial fatty acid markers (e.g. 18:2n-6 and 18:3n-3), consistent with depletions in both bulk and 18:2n-6 $\delta^{13}C$ values in the same month. In June and August, invertebrates were characterized by greater contributions from in-situ algal sources and higher proportions of essential fatty acids (e.g. DHA and EPA). Proportions of bacterial fatty acids were high in deposit-feeding invertebrates and reveal patterns of differential feeding modes across taxa. These results highlight the current role of diverse carbon sources to Arctic coastal food webs, which may change with future warming. Fatty acid biomarkers and stable isotopes are powerful tools for food web studies and in elucidating the complex pathways by which a variety of carbon sources are assimilated by estuarine fauna.

1.1. INTRODUCTION

Current observations in the Arctic show that seasonal patterns in the environment are shifting due to climate change, bringing enhanced inputs of freshwater, terrestrial organic matter, and ice-free areas of open water (Carmack et al., 2006). These changes may lead to shifts in carbon cycling and ecosystem productivity. It was originally thought that terrestrial organic carbon (tOC) is mostly refractory and not an important food source for marine consumers in the Arctic (e.g. Dittmar and Kattner, 2003; Schell, 1983). However, new evidence has shown that the tOC delivered by Arctic rivers during the spring freshet is a labile source that may be important for microbial and metazoan communities within coastal waters (Holmes et al., 2008).

Coastal lagoons and estuaries in the Beaufort Sea are vital habitats for many organisms including many species of migratory birds (Brown, 2006) and fishes (arctic cisco, arctic cod), which feed primarily on epibenthic fauna (polychaetes, mysids, amphipods) that flourish in coastal waters (Dunton et al., 2006). Using stable isotope (SI) analysis, previous work by Dunton et al. (2012) demonstrated the importance of terrestrial carbon inputs as summer food resources for coastal metazoan communities in the Alaskan Beaufort Sea. Specifically, the carbon and nitrogen stable isotopes of benthic and epibenthic fauna indicate a food web dominated by a variety of omnivorous consumers that are highly influenced by terrestrial carbon sources (Dunton et al., 2012). However, much information is still unknown about seasonal variation of carbon sources, including terrestrial and non-terrestrial carbon sources that may be contributing food resources such as *in situ* production (i.e. diatom, dinoflagellate, green algae), bacterial inputs, primary consumers (i.e. copepods), and the nutritional quality (i.e. omega-3s) of fauna as prey items for higher trophic levels.

Fatty acid (FA) biomarkers are often used in trophic studies to understand the linkages between primary producers supporting secondary production, as they are often source-specific and provide longer-term dietary information than gut- and fecal-content analysis (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Connelly et al. (2015) applied FA, stable isotope, and photosynthetic pigment analyses to particulate organic matter (POM) collected from lagoons along the Alaskan Beaufort Sea coast during winter (April), spring freshet (June), and open water (August) and found strong seasonal variability in the sources of FAs in POM. In general, April POM was comprised of highly refractory FAs (e.g. saturated FA (SFA), bacterial FAs) and animal detritus products (e.g. 18:1n-9), with little evidence of autochthonous production (Connelly et al., 2015). June POM contained relatively high amounts of diatom FA markers (∑16:1/16:0), monounsaturated FAs (MUFAs), and variable amounts of terrestrial FAs (∑18:2n-6 + 18:3n-3), indicating that diatom production and terrestrial inputs were both important sources of FAs in POM at that time. In August, POM was characterized by high proportions of polyunsaturated FAs (PUFAs), terrestrial FAs, dinoflagellate markers (e.g. 22:6n-3/20:5n-3; C_{18} PUFA/C_{16} PUFA), and copepod markers. Collectively, results from Connelly et al. (2015) suggest that POM from Beaufort Sea lagoons were highly terrestrial ($\delta^{13}C \leq$ -25‰) throughout the year, but contain seasonally distinct, highly nutritious essential FAs (EFAs; e.g. 22:6n-3 and 20:5n-3) that may contribute to consumer food resources. However, the assimilation of these FAs by invertebrate consumers is yet to be tested. To date, FA profiles of invertebrates from the Beaufort Sea are scarce, (with the exception of Connelly et al., 2014; Wold et al., 2011), and seasonal surveys are even more rare. Further, while more is known about pelagic zooplankton across the Arctic (Mayzaud

and Boutoute, 2015), comparatively little is known about FA profiles of benthic-associated fauna (Graeve et al. 1997, Connelly et al. 2014, Legezynska et al. 2014).

In this study, we investigated the diets of benthic and epibenthic invertebrates collected in Beaufort Sea lagoons and nearshore sites in April, June, and August. We used a combination of FA profiles, FA-stable isotopes (FA-SI), and total lipid-SI to (1) determine the dominant primary producers influencing consumer diets across seasons, (2) identify taxa with high essential FA (EFA) content as nutritious prey items for higher trophic levels, and (3) present data from FA-SI and lipid-SI analyses for elucidating trophic linkages. We hypothesized that the FAs present in consumer tissues would reflect feeding habits, and would coincide with the POM findings of Connelly et al. (2015). However, POM measurements represent a snap-shot of ambient conditions (on the order of days), whereas FA dynamics in tissues can change on the order of weeks to months, depending on food availability and tissue type (McLeod et al., 2013, Chapter 2). Therefore, we anticipated that there could be significant lags associated with the timing of primary production in POM and the build-up of tracer signals within consumers.

1.2. METHODS

1.2.1. Sample collection

Infaunal and epibenthic invertebrates were collected in April, June, and August from sites within lagoons (n=6) and outside lagoons (referred to as *near shore*, n=4) along the Beaufort Sea coast. (Fig. 2.1). Sampling began in August 2011 and ended in August 2013. In April, only one species was collected for lipid analysis, the amphipod *Onisimus glacialis* (n=6), with each sample containing 1-6 individuals per sample, and individuals with lengths between 10-12 mm. All other samples in June and August are noted in Table

1.1. Invertebrates were collected using Ponar grabs (April, June, August) and small beam trawls (June and August), then sorted and washed over a 1 mm sieve. Except for *Calanus hyperbroeus*, collected with a plankton net. Individuals were identified, measured for length, and frozen at -20ºC in Kaktovik, Alaska, USA. Individuals of the same taxa and from the same sampling site were pooled into a single sample (Table 1.1). Samples were kept frozen and transported back to the laboratory at the University of Texas Marine Science Institute (UTMSI) in coolers, usually within 10 days of sample collection. Immediately upon arrival in Texas, samples were placed in glass centrifuge tubes (15 mL), covered with chloroform (2 mL), and stored in N_2 gas at -20ºC until lipid extraction. FAs were determined from a total of 96 samples from 16 taxa. *Onisimus glacialis* samples from the same field program were collected in April (n=4), June (n=2), and August (n=8) for bulk stable isotope analysis, as detailed by Harris et al. (*in prep*).

1.2.2. Lipid extraction and fatty acid analysis

Lipids were extracted from samples in a 2:1:0.5 ratio of chloroform:methanol:water following Parrish (1999), modified from Folch et al. (1957). The animals were ground and homogenized using a Teflon capped metal rod, sonicated, and centrifuged in the chloroform:methanol:water mixture. Lipid extractions were repeated for a total of 3 times per sample. One third (600µl) of the resulting extract was blown dry with N_2 gas, and FAs were transformed to FA methyl esters (FAMEs) by derivatizing samples with BF3-methanol. FAMEs were run on a Shimadzu GC-FID with a ZB-WAX plus column (Phenomenex; 30 m, 0.53 mm id, 1.0 µm film thickness). FA peaks of commercial standards (Supleco COMP 37, BAME, PUFA 1, PUFA 3) were used to identify FA peaks within samples. An internal standard (23:0) was added to each sample to quantify peaks. Fatty acids are expressed proportionally as a percentage of total identified FAs.

Established FA biomarkers were used to identify dietary source, feeding mode, and nutritional quality of invertebrates (e.g. Dalsgaard et al., 2003). The diatom FA marker used herein is the sum of C16 monounsaturates divided by C16 saturates (∑16:1/16:0), when found in ratios >1 (Claustre et al., 1988) Notably, 14:0, 16:3n-4, 16:4n-1 and 20:5n-3 are also potential diatom markers (Léveillé et al., 1997). In contrast, a ratio of 22:6n-3/20:5n-3 >1 can indicate greater contributions of dinoflagellates compared to diatoms in POM. Terrestrial plant contributions can be inferred from C18 Terr FAs (∑18:2n-6 + 18:3n-3) when found in proportions greater than 2.5 in POM (Budge and Parrish, 1998), and from any amount of ∑22:0 + 24:0. The C18 Terr FAs are dominant FAs of terrestrial plants (e.g. sedge and willow) found in the North Slope of Alaska (Ayaz and Olgun, 2000; Hietala et al., 1998; Spetzman, 1959). We refer to these as "terrestrial FAs", but they are also produced by green algae (i.e. Chlorophytes) that are also found in Arctic coastal waters (Jeffrey, 1976; Suzuki et al., 2002; Wilce and Dunton, 2014). Bacterial markers (∑odd-carbon numbered and branched-chain FAs; Graeve et al., 1997) are generally associated with deposit-feeding organisms that consume reworked organic matter and detrital food sources (Legezyńska et al., 2014). The *Calanus* copepod marker is the sum of 22:1 + 20:1 MUFAs (Falk-Petersen et al., 1987). Carnivory can be inferred from high levels of 18:1n-9, as it is a major storage FA in most marine animals (Dalsgaard et al., 2003; Graeve et al., 1997). The ratio of 18:1n-9/18:1n-7 >1 can also distinguish carnivores vs herbivores. Yet, this ratio should be used with caution, as it may also change due to starvation and fluctuating lipid content (Stübing and Hagen, 2003). Nutritional quality of invertebrates as prey items can be inferred from higher levels of PUFA, 20:5n-3, 22:6n-3, and n-3/n-6.

1.2.3. Stable isotope analysis

Stable carbon isotope values of lipids were measured from remaining total lipid extracts (1600µl) from a subset of crustaceans, which included August samples: *Calanus hyperboreus* (n=2), *Gammarus spp.* (n=3), *Monoporeia affins* (n=2), *Mysis relicta* (n=3), *Onisimus glacialis* (n=7), *Pontoporeia femorata* (n=3); June samples: *Onisimus glacialis* (n=3), *Saduria entomon* (n=3); and April samples: *Onisimus glacialis* (n=6). Lipid extracts were blown dry, and resuspended in 100-300µL of chloroform. From the chloroform-lipid extract, 50µL was transferred to a pre-weighed tin capsule containing a small piece of pre-combusted glass fiber filter. The extract was absorbed onto the filter and kept under the fume hood for at least five hours to allow the excess chloroform to evaporate. Prepared samples were analyzed using a Finnigan MAT Delta Plus stable isotope mass spectrometer coupled to a Carlo Erba 1500 elemental analyzer (CE Instruments, NC 2500) at the UTMSI. Lipid stable isotope values are reported in relation to conventional standards Casein (114859) and USGS Isotopic Reference Material Standard 20192 L-Glutamic Acid with a standard deviation of approximately 0.08‰.

Compound-specific isotope analysis (CSIA) was performed using the same aliquot of previously derivatized FAMEs. The FAMEs dissolved in hexane were measured with a GC-combustion-isotope ratio mass spectrometer (GC-C-IRMS) with a BPX70 column (60 m, 0.25 mm O.D., 0.25 µm film; constant flow 1.5mL min^{-1}) at the UC Davis Stable Isotope Facility (University of California, USA). FAMEs were corrected for the addition of the methyl group by measuring the $\delta^{13}C$ value of the BF3-methanol (-51.5‰) used in the derivatization process. The fractional contribution of the methyl group in a FAME depends on its chain length, where **x** is the fractional carbon contribution of the free FA to the ester.

For example, 18:2n-6 has an **x** of 18/19. The corrected $\delta^{13}C$ value of each FA was calculated with the equation (Abrajano et al., 1994):

$$\text{Equation 2.1:}\quad \delta^{13}C_{FA} = \frac{(\delta^{13}C_{FAME} - (1-x) \times \delta^{13}C_{CH3OH}}{x}$$

CSIA values were reported in relation to an internal standard (12:0), with a standard deviation of 0.0‰. Stable isotope ratios for lipid-SI and FA-SI are calculated using δ-notation (Eq. 2.2) relative to the international standard for carbon, Vienna Pee Dee Belemnite (VPDB) ($^{13}C/^{12}C = 0.0112372$).

$$\text{Equation 2.2:}\quad \delta^{13}C\ (‰) = \left(\frac{^{13}C}{^{12}C}\text{Sample} \div \frac{^{13}C}{^{12}C}\text{VPDB} - 1\right) \times 1000$$

1.2.4. Statistical analysis

To test for seasonal differences among taxa collected in June and August, mean values of major FAs (>5% in more than one taxon) were calculated for each species. The seasonal difference (D) was calculated by taking the mean $\%FA_{JUN}$ – mean $\%FA_{AUG}$ for each species. The D values for five peracarida crustaceans available for both seasons (denoted by †; Tables 2.2 and 2.3) were pooled together to calculate a mean D for all five species. The mean D was tested against zero using a one sample t-test. This test was performed for all five major FAs (Tables 2.2 and 2.3).

Principal component analysis (PCA) was used on FA biomarker data from August and June (Fig. 2.4; Fig. 2.7). Only those biomarkers found in all samples were included in the analysis. Before the analysis, FA data was transformed to meet the assumptions of normality using the centered log-ratio 'compositions' package in R Statistical Software. Taxonomic attributions (color coding and labeling) in the PCA plots were done ad hoc.

One-way analysis of variance (ANOVA) was used to test for differences among seasons in *Onisimus glacialis* (1) individual FAs (mean >3% total FA (TFA); Fig. 2.8), (2) FA biomarkers (Fig. 2.9), (3) lipid-SI values and (4) FA-SI values (Fig. 2.10). Post-hoc

pairwise comparisons were used to test for significant differences among seasons. Bulk stable isotope values for *Onisimus glacialis* provided by Harris et al. (*in prep*) were tested for seasonal differences between two months (April and June), using a Welch's t-test.

1.3. RESULTS

1.3.1. Fatty acids

Across all seasons, the most abundant fatty acids for all taxa were 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:5n-3, and 22:6n-3 (Tables 2.2, 2.3). In addition, 14:0 (Marenzellaria wireni in June), 16:3n-4 (Pontoporeia femorata in August), 18:0 (Priapulus caudatus in June), or 18:2n-6 (Onisimus glacialis in April) were greater than 10% of total fatty acids in some species.

1.3.1.1. August

In August, diatom fatty acid markers (∑16:1/16:0) were greater than one for seven species including: *Priapulus caudatus*, *Terrebellides stroemii*, *Mysis relicta*, *Alcyonidium disciforme*, and the amphipods *Onisimus glacialis*, *Atylus carcinatus*, and *Pontoporeia femorata* (Fig. 2.2). Bacterial fatty acid marker ranged from 1.2% for mysids to 18.6% for the isopod *Saduria entomon*, with the majority of taxa containing greater than 5%. *Calanus* copepod markers (∑C_{20} and C_{22} MUFA) were relatively low among taxa, except for the priapulid worm *Halicriptus spinulosus*, the bryozoan *A. disciforme*, and the native taxa, *Calanus hyperboreus*, which were all greater than 5%. The ratio of 22:6n-3/20:5n-3 was greater than 1 in the suspension feeders *A. disciforme* and *Rhizomolgula globularis*, and in the isopod *S. entomon*. The two terrestrial markers (∑18:2n-6+18:3n-3 and ∑22:0+24:0) were comparatively low in most taxa, except for *C. hyperboreus* and *R. globularis* (Fig. 2.3). In general, %PUFA was quite variable, ranging from 6.8% in the amphipod

Monoporeia affins to 45.4% in the bryozoan *A. disciforme*. The n-3/n-6 ratios were highest for cumaceans, mysids, *Priapulus caudatus*, isopods, and the amphipods *Monoculodes sp*.

Of the PCA, the first principle component (PC1) accounted for 42% of the variability in biomarker composition (Fig. 2.4b), and the second principle component (PC2) accounted for 24% (Fig. 2.4c). Despite large variation among taxa, PC1 separated invertebrates by taxa type, with mysids, bryozoans, and cumaceans having negative scores, and copepods, ascidians, isopods, and most amphipods generally having positive scores. Factors important for negative scores in PC1 were %PUFA, %20:5n-3 and %22:6n-3. Factors influencing positive scores were predominantly bacterial fatty acid marker, %SFA, and C18 Terr. For PC2, negative scores were driven predominantly by copepod markers, %22:6n-3, and C18 Terr. Invertebrates on the negative axis of PC2 included predominantly copepods, and suspension feeding bryozoans and ascidians. Invertebrates on the positive axis included mostly cumaceans, mysids, isopods and polychaetes, which was largely driven by diatom markers, %SFA, and %20:5n-3. Amphipods were highly variable across PC1 and PC2, possibly due to high diversity of species with a variety of feeding modes. Interestingly, the priapulid worms *Halicriptus spinulosus* and *Priapulus caudatus* were found on opposite sides of the PCA plot.

1.3.1.2. June

In June, diatom markers were relatively low, with the majority of samples with a value <1 (Fig. 2.5). Bacterial markers were high (means greater than 5%) for all taxa measured. Overall, bacterial fatty acids ranged from 2.9% in mysids to 17.7% in the polychaete *Marenzellaria wireni*. Copepod markers varied across taxa, but were greater than 5% for isopods, the worms *Priapulus caudatus* and *M. wireni*, and some *Onisimus glacialis* samples. The 22:6n-3/20:5n-3 ratio was <1 less than one for all taxa. High levels

of C18 Terr (∑18:2n-6+18:3n-3; >2.5%) were observed for *Gammarus* amphipods, isopods, and worms *P. caudatus* and *M. wireni* (Fig. 5). Correspondingly, terrestrial markers ∑22:0+24:0 were only observed in *Gammarus spp., P. caudatus*, and in isopods. Proportions of PUFA were relatively the same within species collected in June compared to August, ranging from 11.1% in *O. glacialis* to 34.6% in mysids. Levels of n-3/n-6 were comparably lower in June compared to August too, ranging from 0.8 in *Gammarus* amphipods to 7.1 in mysids (Fig. 2.7c).

Of the June PCA, the first principle component (PC1) accounted for 54% of the variability in biomarker composition (Fig. 2.7b), and PC2 accounted for 17% (Fig. 2.7c). PC1 separated invertebrates by species, with amphipods *Gammarus spp.* and *Pontoporeia* femorata, and worms *Priapulus caudatus* and *Marenzellaria wireni* on the negative side and mysids and some isopods on the positive side. The factors important for negative scores in PC1 were bacterial FA, C18 Terr, copepod markers and SFA. Factors influencing positive scores were 20:5n-3, 22:6n-3, and PUFA. For PC2, positive scores were driven almost entirely by diatom markers. Invertebrates on the positive axis of PC2 include isopods, and the amphipods *Onisimus glacialis* and *P. femorata*. Invertebrates on the negative axis included mostly mysids and worms, which were largely driven by PUFA and SFA. Many species did cluster around the origin, indicating variability in the FA profiles within species.

1.3.1.3. Seasonal shifts

Despite low sample sizes for some taxa, seasonal differences between June and August were observed for major fatty acids. Notably, proportions of 18:1n-9 decreased significantly from June to August in peracarida crustaceans (Tables 2.2 and 2.3). General trends in biomarker compositions were observed between invertebrates collected in both

seasons. For instance, n-3/n-6 levels increased from June to August for all species, and diatom markers either increased or stayed the same for most species. Proportions of C18 Terr and levels of copepod markers either decreased or stayed the same.

Seasonal shifts from April, June and August were observed for certain FAs and biomarkers for the amphipod *Onisimus glacialis* (Figs. 2.8 and 2.9). Specifically, proportions of 18:2n-6 were significantly higher in April (8.9±6.3%) compared to June (1.1±0.4%) and August (1.2±0.5%) and proportions of 18:1n-9 were significantly higher in April (21.8±1.9%) compared to August (14.2±3.0%). Likewise, 22:6n-3/20:5n-3 and C18 Terr were significantly higher in April (0.4±0.1% and 10.1±6.9%, respectively) than in August (0.2±0.1% and 1.6±0.7%, respectively. The lower 22:6n-3/20:5n-3 values in August were due to increasing proportions of 20:5n-3 from April to August, as proportions of 22:6n-3 did not change. Further, n-3/n-6 values were significantly lower in April (1.6±1.4%) compared to August (4.8±2.4%). Copepod markers were highest in June, and diatom markers were highest in August (Fig. 2.9).

1.3.2. Stable isotopes

FA-SI values for *Onisimus glacialis* showed general patterns of depleted values in April, enriched values in June, and either enriched or intermediate values in August (Fig. 2.10). Significant differences among seasons were found for 18:0 and 18:2n-6, which were lower in April (-29.8‰ and -36.5‰, respectively) compared to June (-23.2‰ and -30.3‰) and August (-24.4‰ and -32.5‰); and for 18:1n-9 and the mean of all fatty acids measured (∑FA), which were lower in April (-31.1‰ and -30.1‰) compared to June (-25.9‰ and -26.1‰), but similar to August (-28.6‰ and -28.1‰). These results agree with bulk isotope values that were depleted in April (-21.4‰), enriched in June (-18.5‰), and intermediate in August (-19.9‰).

Stable isotope values of total lipid extracts from *Onisimus glacialis* did not change significantly between the three seasons, ranging between -27.9 and -17.1‰ (Fig. 2.11). The total lipid-SI values measured for a subset of crustaceans were also quite variable and ranged from -30.4‰ in *Calanus hyperboreus* to -17.1‰ in *O. glacialis*. While error bars surrounding mean values for individual taxa are large, *C. hyperboreus* stands out has having more depleted isotope values (-30.4‰) than the other species.

1.4. DISCUSSION

1.4.1. Seasonal shifts in fatty acids

Fatty acid profiles and biomarkers were studied in a range of Arctic invertebrate taxa to identify dietary source, feeding mode, and nutritional quality. The majority of samples were collected in June (spring) and August (summer), and reveal seasonal shifts in available food sources. The June-August transition was characterized by an increase in fresh algal food sources, predominantly from diatoms, and an increase in nutritional EFAs. FA profiles and FA-SI values for *Onisimus* amphipods collected in all 3 months showed enhanced importance of terrestrial organic matter sources in April compared to June and August.

1.4.1.1. April

Seasonal shifts in lagoonal food resources were inferred from FA profiles of infaunal and epibenthic invertebrates. Of the invertebrate taxa, the amphipod *Onisimus glacialis* was the only species collected for all three seasons. In these coastal lagoons, dark and cold winter conditions persist from late November to January, with snow-covered sea ice remaining through May or June (Nicolaus et al., 2013). Since little sunlight can reach the waters below, primary production is limited, and new food sources are likely

unavailable for consumers. Enhanced levels of 18:2n-6, 18:3n-3, and 18:1n-9 in *O. glacialis* in April suggest that these amphipods subsist off of terrestrial plant material with tendencies toward carnivory. *Onisimus spp.* are scavengers, known to consume a diet of detritus, carcasses, and plant material (Arndt and Beuchel, 2006). Stomach content analysis of a congeneric species, *O. littoralis*, collected from the Beaufort Sea in April revealed low concentrations of diatom cells and high prevalence of crustacean parts in guts (Carey and Boudrias, 1987; Gradinger and Bluhm, 2010). It was not until late May and June that ice algae (pennate diatoms) were found in the guts of *O. littoralis*. Likewise, diatom FA markers were low in *O. glacialis* (this study) and in POM samples collected in April (Connelly et al., 2015). The high levels of 22:6n-3/20:5n-3 in *O. glacialis* in April likely reflect catabolism of 20:5n-3, not enhanced inputs of 22:6n-3, which do not change seasonally. Studies have demonstrated that 22:6n-3 is more conserved than 20:5n-3 through marine food webs (Carreón-Palau et al., 2013), including hyperbenthic food webs of the Beaufort Sea (Connelly et al., 2014), likely because 20:5n-3 is generally more metabolically active (e.g. eicosanoids) than 22:6n-3 (Tocher, 2010). We hypothesize that FA profiles in winter were more strongly influenced by catabolism of FA rather than direct dietary sources. As more metabolically active PUFAs were catabolized (e.g. 20:5n-3), the less active PUFAs (e.g. 18:2n-6) were retained and remained at higher proportions. Therefore, the dominance of C18 Terr FAs in April partly reflects catabolism of other FAs and consumption of terrestrial matter. The importance of terrestrial food sources is further supported by bulk- and FA-SI values of *O. glacialis* that were more depleted in April compared to later months.

1.4.1.2. June

In June, full daylight persists for 24 hours, and surface air temperatures across the Arctic increase. There is also an abundance of terrestrial organic matter in the lagoons at this time as a consequence of spring runoff (Connelly et al., 2015). Despite the large influx of terrestrial organic matter, however, terrestrial FAs did not become proportionally more important in the tissues of consumers in June as compared to April. Rather, a combination of terrestrial FAs, bacterial FAs, copepod markers, and 18:1n-9 appeared to be important, suggesting that detritivory and carnivory continue to be dominant feeding modes for consumers at this time, especially for benthic worms, amphipods, and isopods. Trophic Level (TL) was calculated for *Onisimus glacialis* collected in April, June and August, using the mean values of $\delta^{15}N$ POM collected in corresponding lagoons where the amphipods were collected within the same month and year (see: Appendix A). A shift in TL of *O. glacialis* was observed from April (2.1) to June (2.7), further supporting the idea of enhanced carnivory for this species. Despite high abundance of diatom markers (∑16:1/16:0 and 20:5n-3) measured in POM (e.g. Connelly et al. 2015), diatom markers were low for all taxa measured in June. Given that incorporation of FAs from diet to tissues occurs with an associated lag-time (e.g. McLeod et al., 2013), the diatom production present in June may not have been fully incorporated in invertebrates collected at the same time period. Rather, the FA profiles in June most likely reflect food sources consumed in late April, and May, before the onset of snow and sea ice melt. It is possible that ice algae are present in these lagoons in months leading up to June, but the contribution to total marine primary production is likely minor (5-10%) (Horner and Schrader, 1982). The lack of diatom markers in FA profiles suggests that ice algae, and even benthic diatoms were not important components of consumer diets in June. Mysids collected in June had higher

levels of 22:6n-3 and 20:5n-3, compared to other taxa. A laboratory study by Schlechtriem et al. (2008) investigated the influence of starvation on the FA profiles of *Mysis relicta*, and found that proportions of 22:6n-3 increased after 3-6 weeks of fasting. Given the high proportions of 22:6n-3, and low levels of diatom, copepod and bacterial markers in *M. relicta* in this study, we conclude that mysids are removed from autochthonous food sources, and may be experiencing a period of fasting in months leading up to June.

1.4.1.3. August

In August, water and atmospheric conditions are relatively warm, with no sea ice present. While most of the river discharge into the Beaufort Sea has slowed (McClelland et al., 2014), large amounts of terrestrial FA markers (18:2n-6, 18:3n-3, 22:0, 24:0) remain in the POM pool (Connelly et al., 2015). Indeed, the terrestrial signal in the POM is proportionally highest during August. While total amounts of terrestrial and locally produced POM peak in the spring, contributions from in-situ production decrease more than terrestrial contributions as the summer progresses (Connelly et al., 2015). In contrast, invertebrate FA profiles during August were low in terrestrial FA markers for all species except the suspension feeders *Rhizomogula globularis* (ascidian) and *Calanus hyperboreus* (copepod). The ascidian is a non-selective filter feeder, and in the coastal Beaufort Sea, is known to have depleted bulk carbon isotope values (-25‰), indicative of terrestrial diet sources (e.g. Dunton et al., 1989; Dunton and Schell, 1987). The copepod is considered an herbivore (Hobson et al., 2002), and collections of *C. hyperboreus* from the Beaufort Sea shelf in summer has shown to have depleted carbon isotope values (-24‰) (e.g. Connelly et al., 2014; Harris et al. *in prep*). Therefore, the presence of terrestrial FA markers in these taxa are consistent with existing knowledge of their feeding ecology and stable isotope data, and may represent an important food web link between terrestrial producers and

marine consumers. Alternatively, lower carbon isotope values in *C. hyperboreus* could result from high total lipid content and endogenous production of wax esters, as discussed in section 1.4.3.

Diatoms were an important component of invertebrate diets in August for a number of taxa, including: priapulid worms, amphipods, mysids and polychaetes, which ultimately contribute to higher levels of EFAs such as 20:5n-3. Connelly et al. (2015) reported a shift from diatom markers to dinoflagellate markers in POM between June and August. Yet, most invertebrate profiles reflect a dominance of diatoms. As mentioned, there may be a lag between available food sources and incorporation by consumers. Therefore, August FA profiles may reflect feeding in June and July. Legezyńska et al. (2014) also documented high amounts of diatom FAs in benthic invertebrates collected in summer fjords. In our study, only a few species (*Alcyonidium disciforme, Saduria entomon,* and *Rhizomogula globularis*) had 22:6n-3/20:5n-3 ratios >1, which might indicate a dominance of dinoflagellates in the diet. However, 22:6n-3 is also known to increase with trophic level for hyperbenthic invertebrates in the Beaufort Sea (Connelly et al., 2014), as also seen elsewhere (Carreón-Palau et al., 2013), which is probably the case of the isopod *S. entomon*. The bryozoan and ascidian are both suspension feeders, and likely have a stronger link to pelagic production (i.e. dinoflagellates) than benthic production (i.e. benthic microalgae). Little is known about the feeding ecology of the suspension feeder *A. disciforme*, the only free-living Arctic bryozoan (Kukliski and Porter, 2004), and FA profiles from this study may provide the first look into their dietary sources. The high amount of FA biomarkers indicative of copepods, dinoflagellates, and bacteria reflects a polytrophic feeding style.

Bacteria make important contributions in microbial food webs, typical of stratified and nutrient depleted areas such as coastal lagoons (Cushing, 1989). Bacterial FAs found at high levels during August in the amphipods *Pontoporeia femorata* and *Monoporeia affins*, and the polychaete *Terrebellides stroemi* are consistent with their deposit feeding behavior (Legezyńska et al., 2014). The isopod *Saduria entomon* is a scavenger and predator, known to feed selectively on the amphipod *M. affins* (syn. *Pontoporeia affins*, e.g. Baltic Sea, Ejdung and Elmgren, 2001; Leonardsson, 1991). Thus, it is possible that high bacterial FAs in *S. entomon* are due to predation on deposit-feeding amphipods, and not directly on detrital material.

1.4.2. Fatty acid-stable isotopes

FA-$\delta^{13}C$ measurements for the amphipod *Onisimus glacialis* revealed shifts from depleted values in April to enriched values in June and August. Goñi et al. (2005) measured the FA-$\delta^{13}C$ values of organic carbon in suspended sediments and surface sediments from the Mackenzie River and shelf in the eastern Beaufort Sea. River suspended sediments contained FAs with highly depleted $\delta^{13}C$ values (-36‰ to -40‰), indicative of C3 vascular plants and/or other freshwater primary producers. Shelf sediments contained FAs with more enriched values (-26‰ to -30‰). Given these sources, *O. glacialis* FA-SI values in April displayed a mixture of marine and terrestrial sources, ranging from -25‰ (15:0) to -38‰ (18:3n-3), with June and August having stronger influence from marine production.

Although generally referred to as terrestrial FA markers, 18:2n-6 and 18:3n-3 are also synthesized by green algae (Dalsgaard et al., 2003; Dunstan et al., 1992). Field studies have shown that green algae can be found in high amounts in Arctic waters (Jeffrey, 1976; Morata et al., 2008; Suzuki et al., 2002; Wilce and Dunton, 2014). Compound-specific isotope data of green algae in the Arctic is scarce, but studies from other cold water

environments may be useful for distinguishing between terrestrial and algal sources. Field measurements of primary producers in Trinity Bay, Newfoundland found that the terrestrial plant *Equisetum sp.* had 18:2n-6 and 18:3n-3 values at approximately -33‰ (Budge et al., 2001), whereas marine green algae had 18:3n-3 values of approximately -26‰ in the North Sea (e.g. Scheldt estuary, Boschker et al., 2005). In *O. glacialis*, significant changes from lower 18:2n-6 $\delta^{13}C$ values in April to higher $\delta^{13}C$ 18:2n-6 values in June and August are consistent with a shift from a predominantly terrestrial source in April, to a mixture of terrestrial and green algal sources in later months. Although 18:3n-3 was not detected in June and August in all samples to allow for robust statistical analysis, measured values for all three months were less than -33‰. Overall, the FA-SI trends indicate that terrestrially derived carbon is proportionally most important for overwintering amphipods, while *in situ* marine production is proportionally dominant during spring and summer.

Recent studies have tried to characterize the FA-SI values of ice algae and pelagic phytoplankton using common diatom markers such as 20:5n-3. Ice algae collected near Barrow, Alaska, USA have been reported to have higher 20:5n-3 values (-18‰) compared to pelagic algae (-27‰) (e.g. Budge et al., 2008). Likewise, ice algae in the Bering Sea had higher 20:5n-3 values (-27‰) compared to pelagic algae below the ice (-30‰) (e.g. Wang et al., 2014a). While absolute FA-SI signatures are not possible to obtain, due to environmental variation, in general these studies have shown a carbon enrichment in ice algae relative to pelagic phytoplankton, most likely due to the DIC-limited, semi-enclosed ice environment in which they grow (Smith, 2012). *O. glacialis* 20:5n-3 values in our study were not significantly different across seasons, although slight increases were observed in June (-26‰) compared to August and April (-28‰). It is likely that 20:5n-3 came from the same source across seasons, but this source cannot be identified with the available data.

Future field studies hoping to quantify ice algae vs pelagic phytoplankton contributions to diets should make FA-SI measurements of POM a priority.

1.4.3. Total lipid stable isotopes

We provide the first bulk lipid SI data for Arctic crustaceans. Variation in $\delta^{13}C$ among and within taxa suggests that total lipid isotopes could be used to track the flow of lipids within a food web, given distinct endmember sources. While this is not possible with our current sample size, some interesting trends were found. The most negative values (-30‰) were observed in *Calanus hyperboreus*, which may be the result of endogenous lipid production. Herbivorous copepods (e.g. *C. hyperboreus)* contain up to 85% lipids per total body mass (Vogedes et al., 2010) and synthesize high levels of wax esters, particularly in the form of long-chain MUFAs (20:1n-9 and 22:1n-11) (Graeve et al., 2005). At least half of the wax ester pool (comprising 85-95% of lipids in *C. hyperboreus*) are not derived from dietary lipids, but are produced *de novo* from protein and carbohydrate precursors (Falk-Petersen et al., 2009). We speculate that the high amount of endogenous production may result in depleted lipid isotope values. Because none of the other crustaceans have values similar to *C. hyperboreus* suggests that their lipid stores did not derive from copepod lipids. This agrees with the biomarker data for peracarida crustaceans which all had copepod markers less than 5%. Interestingly, von Biela et al. (2012) observed high amounts of copepods in the guts of year-of-young Arctic cisco (*Coregonus autumnalis*), with unusually negative bulk carbon SI values (-26‰), thought to be attributed to terrestrial carbon sources. We speculate that the negative isotope values may have resulted from a *Calanus*-rich diet with high amounts of lipids derived from a combination of terrestrial carbon and endogenously produced wax esters.

It is well known that high amounts of lipids, that are naturally carbon depleted, can alter the bulk carbon isotope value of an organism, and equations have been created to mathematically correct for this "lipid bias" (e.g. Post et al., 2007). Based on our total lipid-SI results, it is clear that not all lipids are created equal, and such correction equations should be used with caution. Ideally, species-specific lipid corrections could be created (e.g. Mohan et al. *In Review*), but such methods are often impractical for wide-ranged food web studies. Instead, we encourage researchers to not "correct" for lipid-bias, but rather to acknowledge the possible effects lipids have on bulk isotope values, especially in lipid-rich tissues (e.g. livers, blubber). In polar regions especially, the flux of energy within food webs is lipid-driven (Møller, 2006). By removing or correcting for lipids, valuable information about trophic sources may be lost.

However, much is still unknown about the use of lipid-SIs to trace food webs. Because our study was limited by sample size, strong conclusions cannot be made. Future studies should explore the lipid-SI linkages between lipid-rich prey and lipid-rich consumers (e.g. copepods, Arctic cod, seals, whales, polar bears).

1.4.4. Nutritional content of prey for higher consumers

Invertebrates with high 20:5n-3, 22:6n-3, and n-3/n-6 ratios are essential prey items for fish, birds, and mammals because these EFA are required for growth, reproduction, survival, and pigmentation (Parrish, 2009; Sargent et al., 1999). In general, specimens collected in August had higher amounts of EFA and n-3 PUFA than those collected in June. Regardless of season, mysids, isopods and some amphipod species (*Onisimus glacialis, Atylus carcinatus, Monoculodes sp.*) were the most nutritious in this regard. These particular prey species (mysids and amphipods) are also known to be the principal prey items found in stomachs of demersal fish, like Arctic cod (*Boreogadus saida*) collected in

nearshore waters (Craig et al., 1982). Arctic cod represent a critical trophic link between lower trophic levels and top predators such as whales, seals, and seabirds, and their dietary sources change seasonally (Bradstreet, 1986; Hobson et al., 2002). Arctic cod have been shown to consume amphipods and copepods during ice-covered periods while copepods and mysids are their primary food source during warmer ice-free seasons (Benoit et al., 2010; Bradstreet and Cross, 1982). Our study has shown that terrestrial carbon sources are important for sustaining invertebrate communities (especially amphipods and copepods) throughout the year, when autochthonous sources are scarce, and in turn, might influence the population dynamics of fish like Arctic cod in these near-shore systems. Therefore, not only does the nutritional content of prey (e.g. EFA) affect Arctic cod feeding, but terrestrial FAs are indirectly important as subsistence sources for prey. Our results may also provide insight into the dietary influences of other important Arctic species such as bowhead whales (*Balaena mysticetus*), which are known to feed heavily on copepods and mysids (Lowry and Burns, 1980; Pomerleau et al., 2010).

1.5. CONCLUSIONS

Fatty acid biomarkers are useful tools for elucidating trophic linkages across food webs, and their use is strengthened by a multi-proxy approach with other trophic indicators such as stable isotope and gut content analyses. The high seasonal variability of dietary sources in the Arctic often leads to rapid diet changes that are not always observed by sampling in one season (Kaufman et al., 2008). We observed a coastal lagoon food web that shifted from subsistence and carnivorous diets in April and June, to a labile *in situ* dietary source in August, predominately driven by diatom blooms that occurred a few months before sampling. High relative levels of terrestrial FAs in April highlight the importance of tOM inputs for sustaining organisms throughout winter, whereas inputs from

primary producers rich in EFAs during the growing season are crucial for rapidly accumulating energy stores for a variety of physiological uses. The results presented herein provide a baseline understanding of seasonal trophic linkages in Beaufort Sea lagoons. As the advancement of climate change and coastal development in the Arctic progresses, it is becoming increasingly important to compare past and current physical, chemical, and biological drivers to anticipate future changes.

Table 1.1. Species information and sites where animals were collected from nearshore locations and coastal lagoons of the Alaskan Beaufort Sea coast. Lagoons sites include: Angun (AN), Demarcation Bay (DE), Jago (JA), Kaktovik (KA), Nuvagapak (NU), and Tapkaurak (TA). Nearshore sites include: Bernard Spit (BE), Bernard Point (BP), Demarcation Point (DP), and the Hulahula Delta (HU). '*n*' is the number of samples per species for each month. Indiv/sample is the range in the number of individuals pooled in each sample. Mean length (mm) is the range in the lengths of individuals in each sample. Trophic level is reported where available, as determined by Harris et al. (*in prep*) from species collected in August from four lagoons (KA, JA, AN, NU). Trophic guild denotes feeding mode (De = deposit, Om = omnivore, Su = suspension). Table excludes *Onisimus* amphipods collected in April; see methods for details.

Table 1.1.

	Species	Site	June			August			Trophic Level	Trophic Guild
			n	Indiv/ sample	Length (mm)	*n*	Indiv/ sample	Length (mm)		
Annelida										
Spionida	*Marenzellaria wireni*	AN	2	5,7	17-22	-	-	-	-	De
Terrebellida	*Terrebellides stroemii*	KA	-	-	-	2	2,5	16-17	1.7	De
Arthropoda										
Amphipoda	*Atylus carinatus*	JA, KA	-	-	-	3	1-4	14-20	1.6	Om
Amphipoda	*Gammarus spp.*	AN, NU, TA	2	2	14,15	3	1	14-21	1.5	Om
Amphipoda	*Monoculodes sp.*	BE, JA	-	-	-	2	3,6	9-10	-	Om
Amphipoda	*Monoporeia affins*	JA, NU, TA	-	-	-	3	1-16	8-11	1.6	De
Amphipoda	*Onisimus glacialis*	AN, BE, BP, DE, DP, HU, JA, KA, NU, TA	3	1-6	9-10	7	1-27	7-14	1.8	Om
Amphipoda	*Pontoporeia femorata*	KA, NU, TA	1	5	10	4	1-8	6-9	1.5	De
Cumacea	*Diastylis goodsiri*	KA	-	-	-	1	25	9	2.4	Om
Isopoda	*Saduria entomon*	AN, JA, KA, NU	8	1-5	8-25	1	1	15	2.3	Om
Mysidacea	*Mysis relicta*	AN, BE, BP, DE, DP, HU, JA, KA, NU, TA	3	2-8	19-25	30	1-25	10-50	1.6	Om
Copepoda	*Calanus hyperboreus*	BE, JA	-	-	-	2	6-11	3-4	2	Su
Bryozoa										
Ctenostomata	*Alcyonidium disciforme*	JA	-	-	-	3	1-2	25-39	1.8	Su
Cephalorhyncha										
Priapulida	*Halicriptus spinulosus*	AN	-	-	-	1	3	12	2.3	De
Priapulida	*Priapulus caudatus*	BE, JA	2	2-6	8-20	1	4	23	2.4	De
Chordata										
Stolidobranchia	*Rhizomolgula globularis*	KA, TA	-	-	-	6	1-2	15-22	-	Su

Table 1.2. Major fatty acids (% of total fatty acids; >5% in more than one taxon) for species collected in August from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast. Standard deviation is reported in parenthesis, except when $n = 2$ and is reported as half range. '*n*' is the number of samples analyzed for fatty acid proportions. Taxa type are noted as follows: amphipod (Am), ascidian (As), bryozoan (B), copepod (Co), cumacean (Cu), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). (†) Denotes species collected in both June and August.

August	Species	*n*	16:0		16:1n-7		18:1n-9		18:1n-7		20:5n-3		22:6n-3		Type
Annelida															
Terrebellida	*Terrebellides stroemii*	2	20.2	(0.0)	20.4	(0.4)	3.1	(0.0)	10.6	(0.8)	13.1	(0.1)	1.9	(0.0)	Po
Arthropoda															
Amphipoda	*Atylus sp.*	3	21.1	(2.5)	20.2	(2.3)	14.6	(0.7)	5.3	(0.4)	12.2	(3.7)	3.7	(0.9)	Am
Amphipoda	*†Gammarus sp.*	3	27.5	(4.6)	21.2	(14.8)	15.5	(5.5)	4.2	(0.5)	6.6	(2.3)	2.0	(1.1)	Am
Amphipoda	*Monoculodes sp.*	2	25.0	(0.4)	10.7	(2.9)	10.5	(0.8)	8.9	(0.4)	14.0	(1.9)	7.1	(3.4)	Am
Amphipoda	*Monoporeia affins*	2	27.3	(3.2)	15.9	(3.3)	15.6	(7.7)	3.6	(1.1)	5.4	(2.7)	1.9	(0.6)	Am
Amphipoda	*†Onisimus sp.*	7	21.6	(5.8)	25.0	(7.6)	14.0	(2.8)	4.4	(0.9)	10.4	(4.2)	2.1	(1.1)	Am
Amphipoda	*†Pontoporeia femorata*	4	24.0	(4.2)	23.4	(6.2)	10.8	(1.2)	4.0	(2.1)	4.0	(1.6)	0.7	(0.4)	Am
Cumacea	*Diastylis goodsiri*	1	19.1	-	11.8	-	6.6	-	14.4	-	21.5	-	2.9	-	Cu
Isopoda	*†Saduria entomon*	1	28.0	-	10.5	-	6.9	-	6.0	-	2.2	-	9.3	-	I
Mysidacea	*†Mysis relicta*	30	23.2	(3.8)	21.1	(7.2)	11.9	(1.9)	4.3	(1.0)	14.3	(4.4)	6.8	(3.7)	M
Copepoda	*Calanus hyperboreus*	2	17.1	(1.2)	15.0	(0.3)	3.7	(0.8)	1.7	(0.4)	8.2	(1.6)	4.7	(0.8)	Co
Bryozoa															
Ctenostomata	*Alcyonidium disciforme*	3	12.5	(1.7)	11.9	(4.8)	3.6	(0.7)	1.9	(0.3)	11.6	(1.4)	15.7	(2.8)	B
Cephalorhyncha															
Priapulida	*Halicriptus spinulosus*	1	16.0	-	7.0	-	4.9	-	13.3	-	9.4	-	1.4	-	Pr
Priapulida	*†Priapulus caudatus*	1	13.0	-	21.5	-	2.1	-	16.3	-	21.9	-	1.2	-	Pr
Chordata															
Stolidobranchia	*Rhizomogula globularis*	6	25.1	(6.6)	12.6	(4.2)	3.6	(0.7)	8.5	(0.9)	8.3	(4.8)	5.7	(1.7)	As

Table 1.3. Major fatty acids (% of total fatty acids; >5% in more than one taxon) for species collected in June from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast. Standard deviation is reported in parenthesis, except when $n = 2$ and is reported as half range. '*n*' is the number of samples analyzed for fatty acid proportions. Taxa type are noted as follows: amphipod (Am), ascidian (As), bryozoan (B), copepod (Co), cumacean (Cu), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). (†) Denotes species collected in both June and August.

June	**Species**	***n***	**16:0**		**16:1n-7**		**18:1n-9**		**18:1n-7**		**20:5n-3**		**22:6n-3**		**Type**
Annelida															
Spionida	*Marenzellaria wireni*	2	15.2	(1.4)	5.0	(0.4)	4.0	(0.6)	6.4	(1.1)	2.8	(1.1)	0.3	(0.2)	Po
Arthropoda															
Amphipoda	†*Gammarus sp.*	2	24.6	(3.0)	14.7	(2.4)	20.2	(1.2)	5.6	(0.1)	3.1	(0.8)	0.9	(0.0)	Am
Amphipoda	†*Onisimus sp.*	3	21.3	(4.6)	22.2	(8.7)	17.9	(7.3)	4.4	(0.6)	8.2	(4.5)	2.3	(1.6)	Am
Amphipoda	†*Pontoporeia femorata*	1	25.9	-	25.9	-	11.8	-	4.7	-	4.2	-	0.7	-	Am
Isopoda	†*Saduria entomon*	8	17.3	(3.3)	12.8	(3.4)	12.6	(3.1)	10.7	(2.3)	7.8	(3.3)	1.7	(0.8)	I
Mysidacea	†*Mysis relicta*	3	31.5	(8.4)	8.9	(2.4)	16.3	(1.0)	4.6	(0.1)	11.5	(5.8)	8.6	(5.1)	M
Cephalorhyncha															
Priapulida	†*Priapulus caudatus*	2	17.2	(0.5)	6.6	(2.4)	2.3	(0.2)	12.3	(0.8)	4.7	(0.7)	0.6	(0.0)	Pr

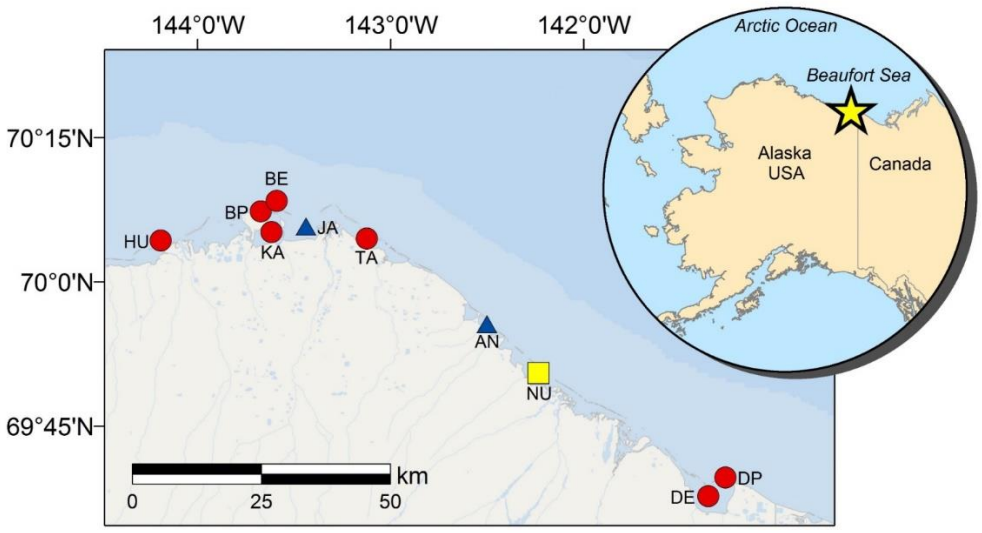

Figure 1.1. Location of lagoon and nearshore sites sampled for invertebrate zooplankton. Squares indicate sites sampled in all 3 seasons (April, June, August). Triangles indicate sites sampled in two seasons, and circles indicate sites sampled in one season (all in August, except for BP in April). Lagoons sites include: Angun (AN), Demarcation Bay (DE), Jago (JA), Kaktovik (KA), Nuvagapak (NU), and Tapkaurak (TA). Nearshore sites include: Bernard Spit (BE), Bernard Point (BP), Demarcation Point (DP), and the Hulahula Delta (HU).

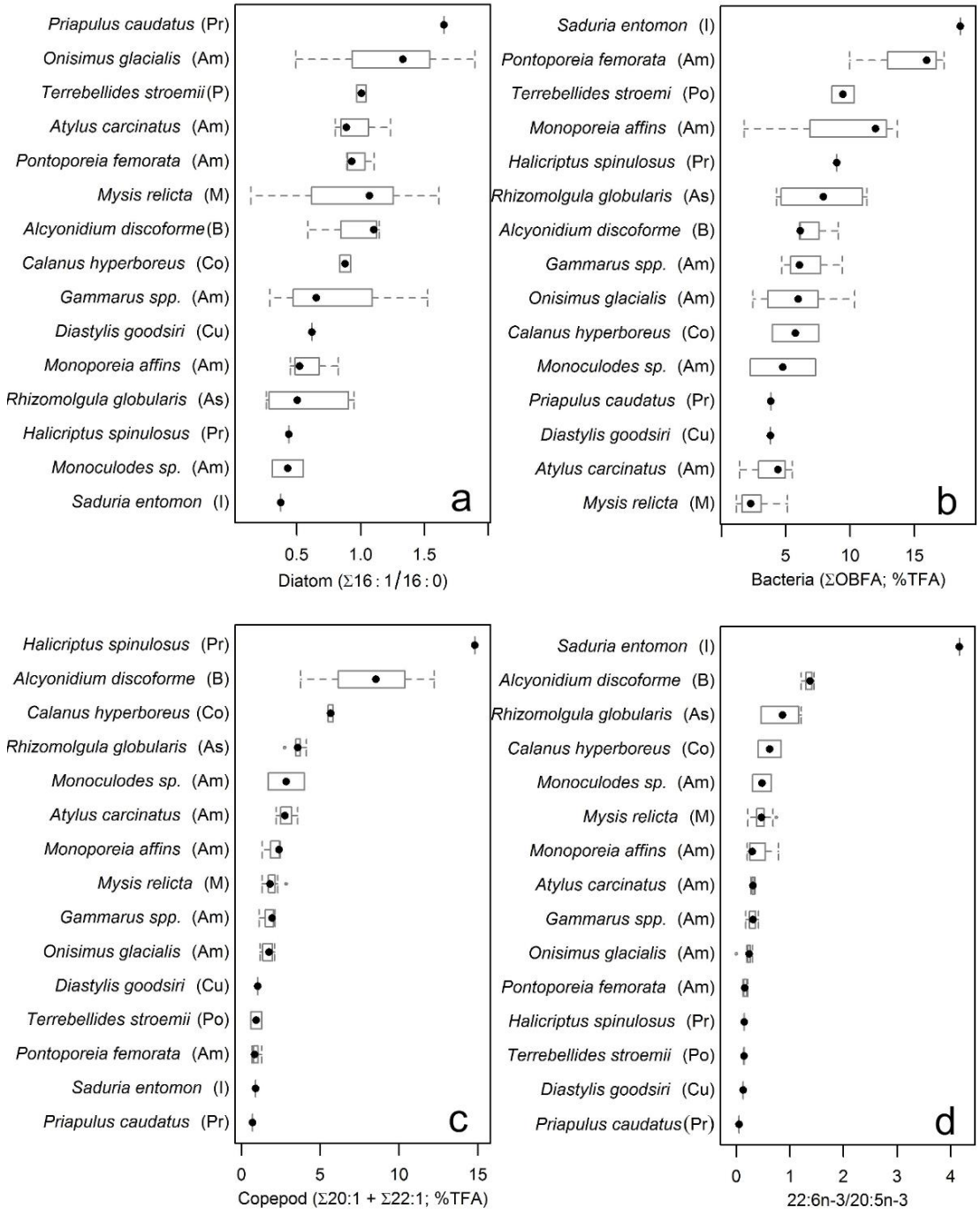

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Figure 1.2. Diatom fatty acid marker (a), bacteria fatty acid marker (b), Copepod fatty acid marker (c), and 22:6n-3/20:5n-3 (d) values for invertebrates collected in August from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast ordered by taxa from highest to lowest. Box and whisker plots show the median (black circle), 25^{th} and 75^{th} percentile (left and right of box). The dashed vertical line is 1.5 times the interquartile range of data. Outliers not within this range are drawn as individual points. Taxa type are noted as follows: amphipod (Am), ascidian (As), bryozoan (B), copepod (Co), cumacean (Cu), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). Bacteria and Copepod markers are % of total fatty acids (%TFA) and Diatom and 22:6n-3/20:5n-3 are unitless ratios. OBFA = odd-carbon numbered and branched chain fatty acids.

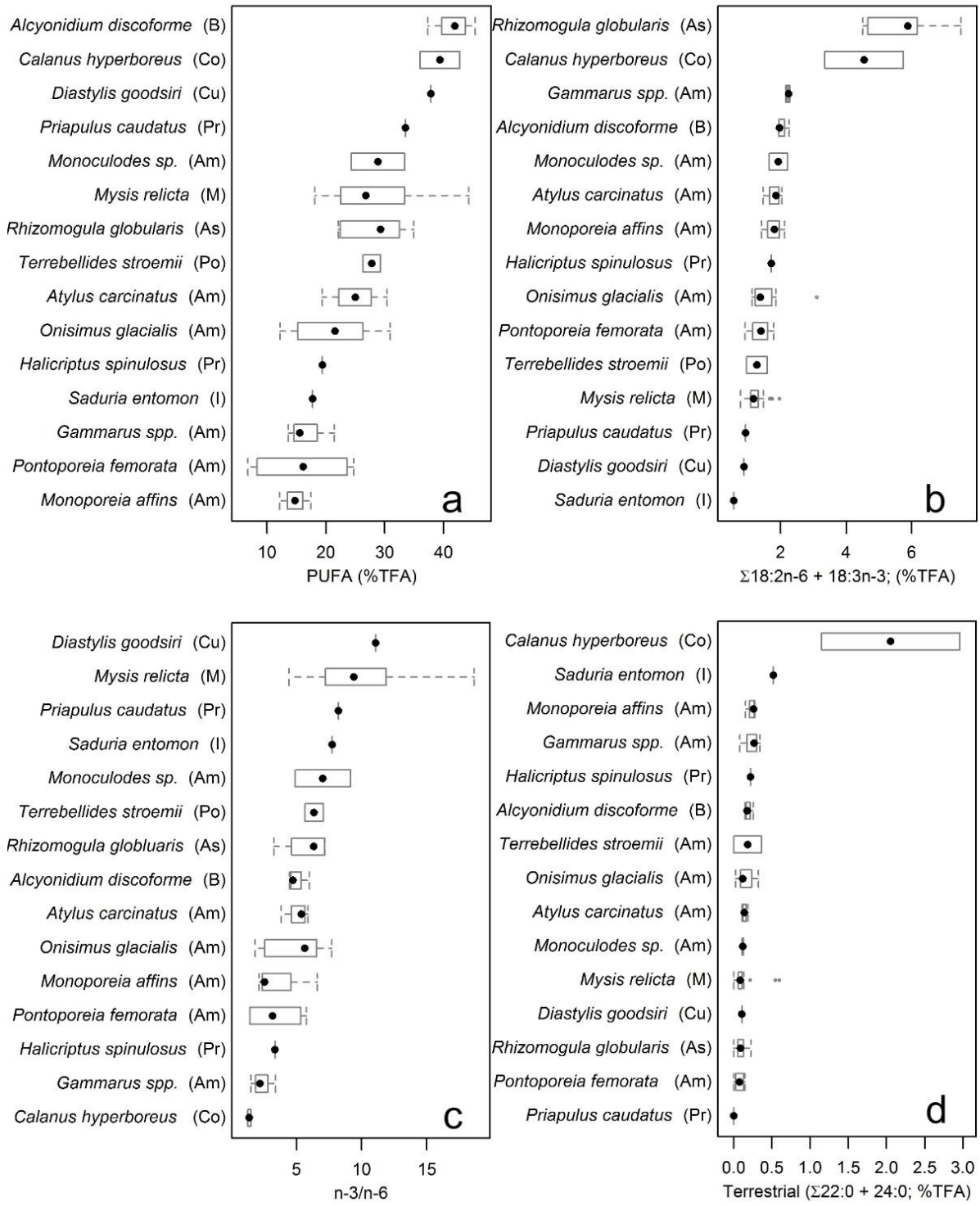

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Figure 1.3. Polyunsaturated fatty acids (PUFA) (a), C_{18} terrestrial FA (18:2n-6 + 18:3n-3) (b), n-3/n-6 (c), and terrestrial FA (22:0 + 24:0) (d) values for invertebrates collected in August from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast ordered by taxa from highest to lowest. See Fig. 1.2 for definitions of box and whiskers. Taxa type are noted as follows: amphipod (Am), ascidian (As), bryozoan (B), copepod (Co), cumacean (Cu), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). PUFA, C_{18} terrestrial, and terrestrial FA (22:0 + 24:0) are % of total fatty acids (%TFA) and n-3/n-6 is a unitless ratio.

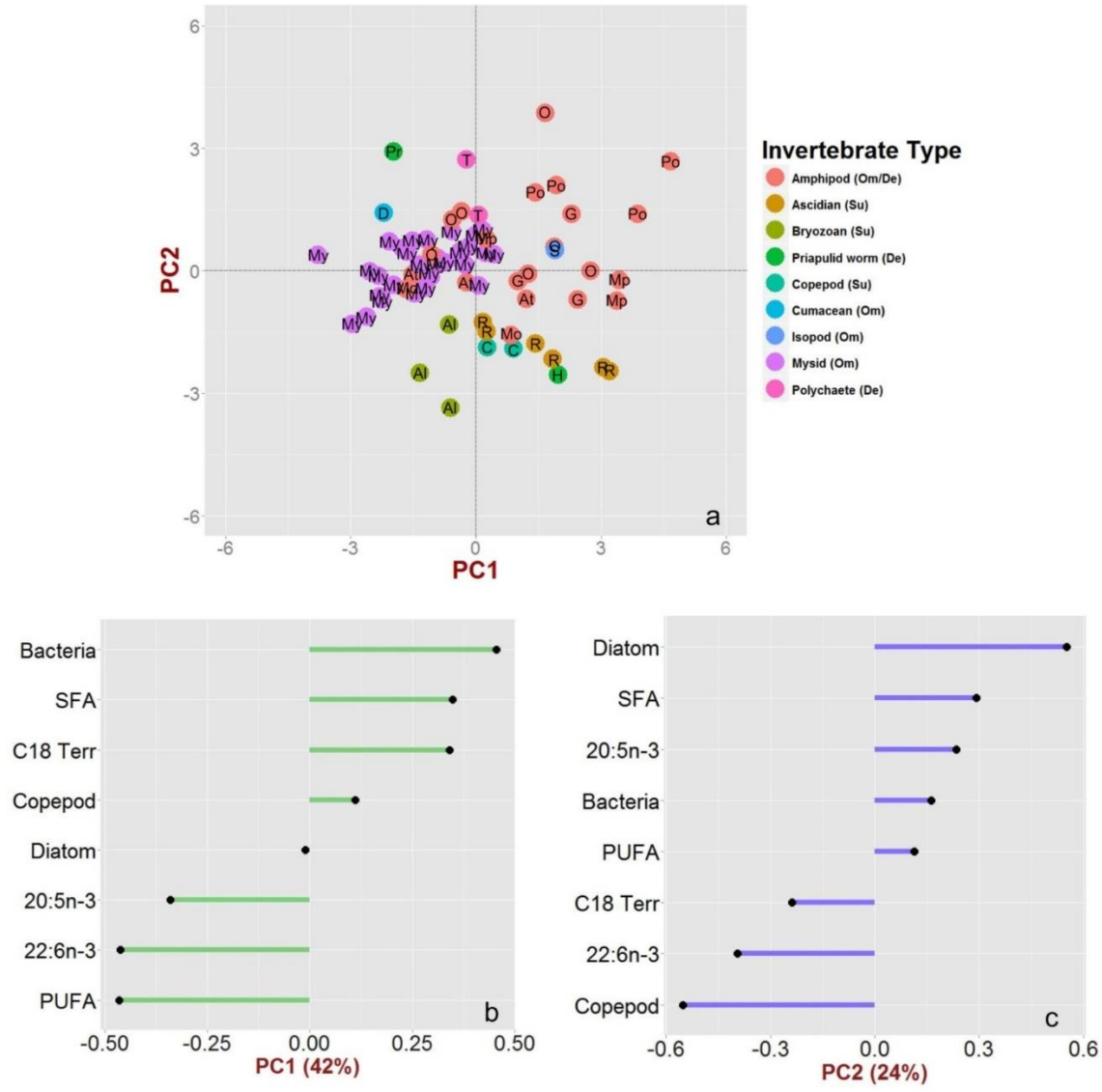

Figure 1.4. Scores (a) from the August principle component analysis for FA biomarkers in invertebrates collected from Alaskan Beaufort Sea nearshore sites and lagoons, and loadings of variables on PC1 (b) and PC2 (c). Taxa are colored based on taxonomic type. Species names (a) are labeled as follows: *Alcyonidium disciforme* (Al), *Atylus carinatus* (At), *Calanus hyperboreus* (C), *Diastylis goodsiri* (D), *Gammarus spp.* (G), *Halicriptus spinulosus* (H), *Monoculodes sp.* (Mo), *Monoporeia affins* (Mp), *Mysis relicta* (My), *Onisimus glacialis* (O), *Pontoporeia femorata* (Po), *Priapulus caudatus* (Pr), *Rhizomolgula globularis* (R), *Saduria entomon* (S), *Terrebellides stroemii* (T).

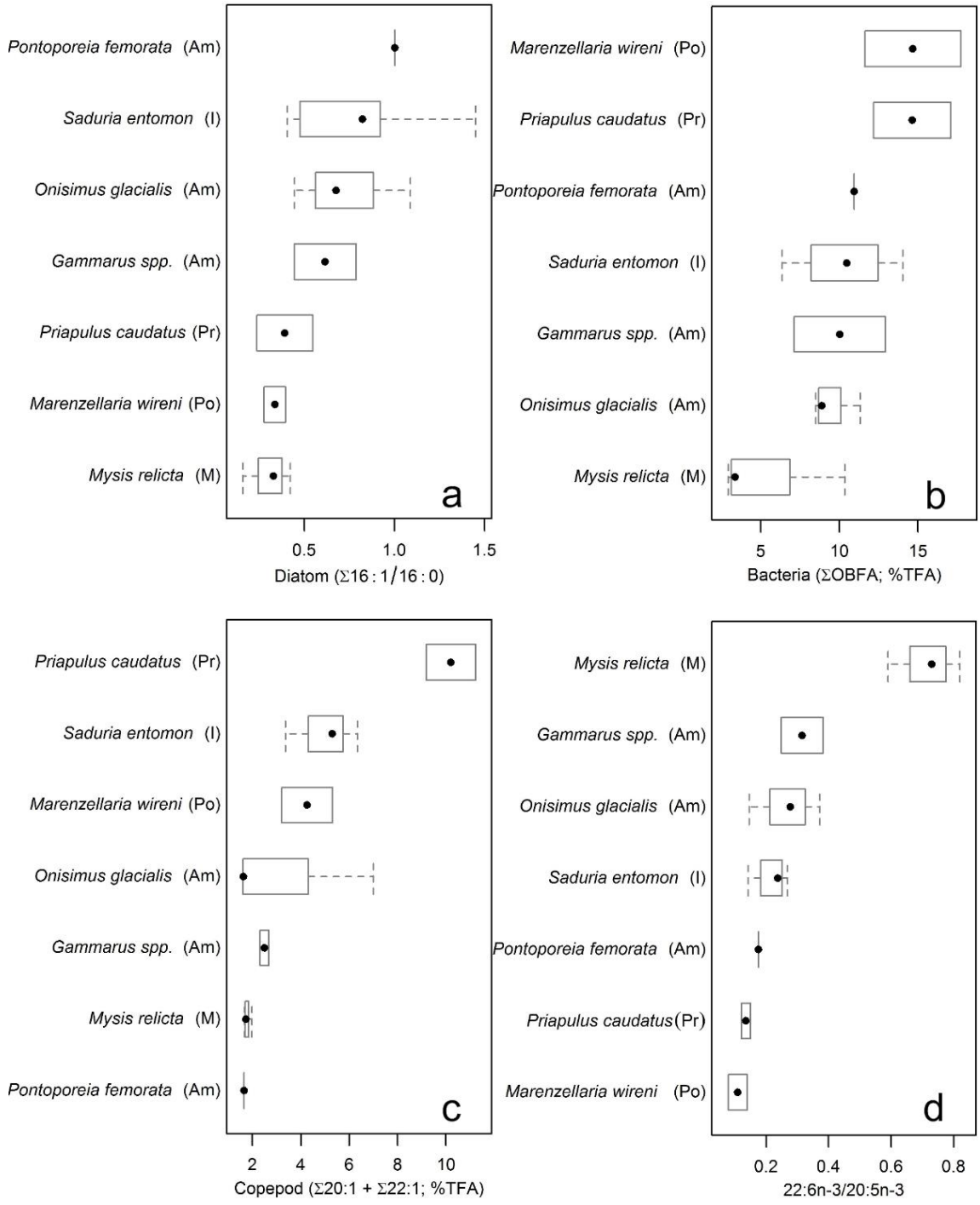

Figure 1.5 continued on next page.

Figure 1.5. Diatom fatty acid marker (a), bacteria fatty acid marker (b), Copepod fatty acid marker (c), and 22:6n-3/20:5n-3 (d) values for invertebrates collected in June from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast ordered by taxa from highest to lowest. See Fig. 1.2 for definitions of box and whiskers. Taxa type are noted as follows: amphipod (Am), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). Bacteria and Copepod markers are % of total fatty acids (%TFA) and Diatom and 22:6n-3/20:5n-3 are unitless ratios. OBFA = odd-carbon numbered and branched chain fatty acids.

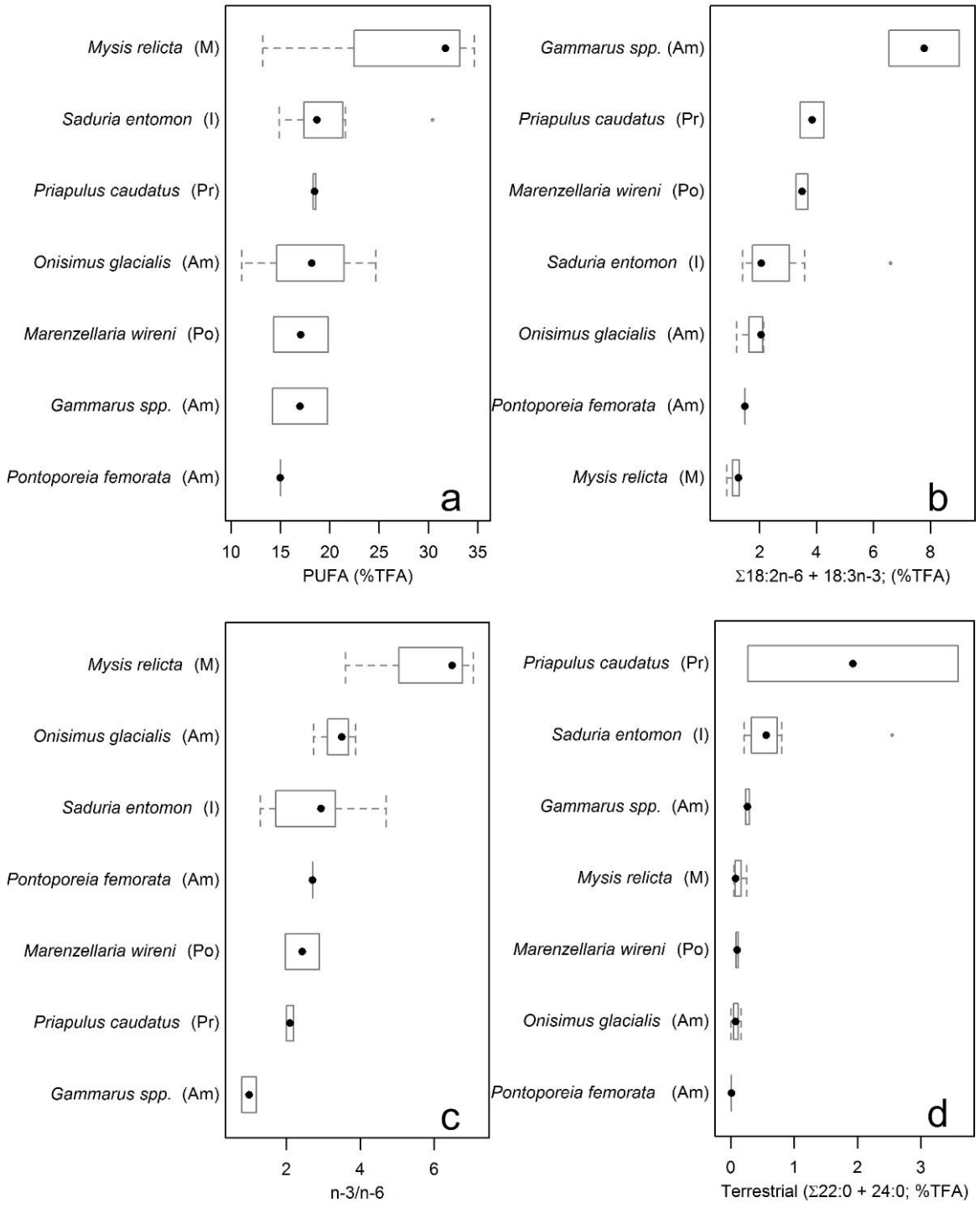

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Figure 1.6. Polyunsaturated fatty acids (PUFA) (a), C_{18} terrestrial FA (18:2n-6 + 18:3n-3) (b), n-3/n-6 (c), and terrestrial FA (22:0 + 24:0) (d) values for invertebrates collected in June from nearshore sites and coastal lagoons of the Alaskan Beaufort Sea coast ordered by taxa from highest to lowest. See Fig. 1.2 for definitions of box and whiskers. Taxa type are noted as follows: amphipod (Am), isopod (I), mysid (M), polychaete (Po), priapulid (Pr). PUFA, C_{18} terrestrial, and terrestrial FA (22:0 + 24:0) are % of total fatty acids (%TFA) and n-3/n-6 is a unitless ratio.

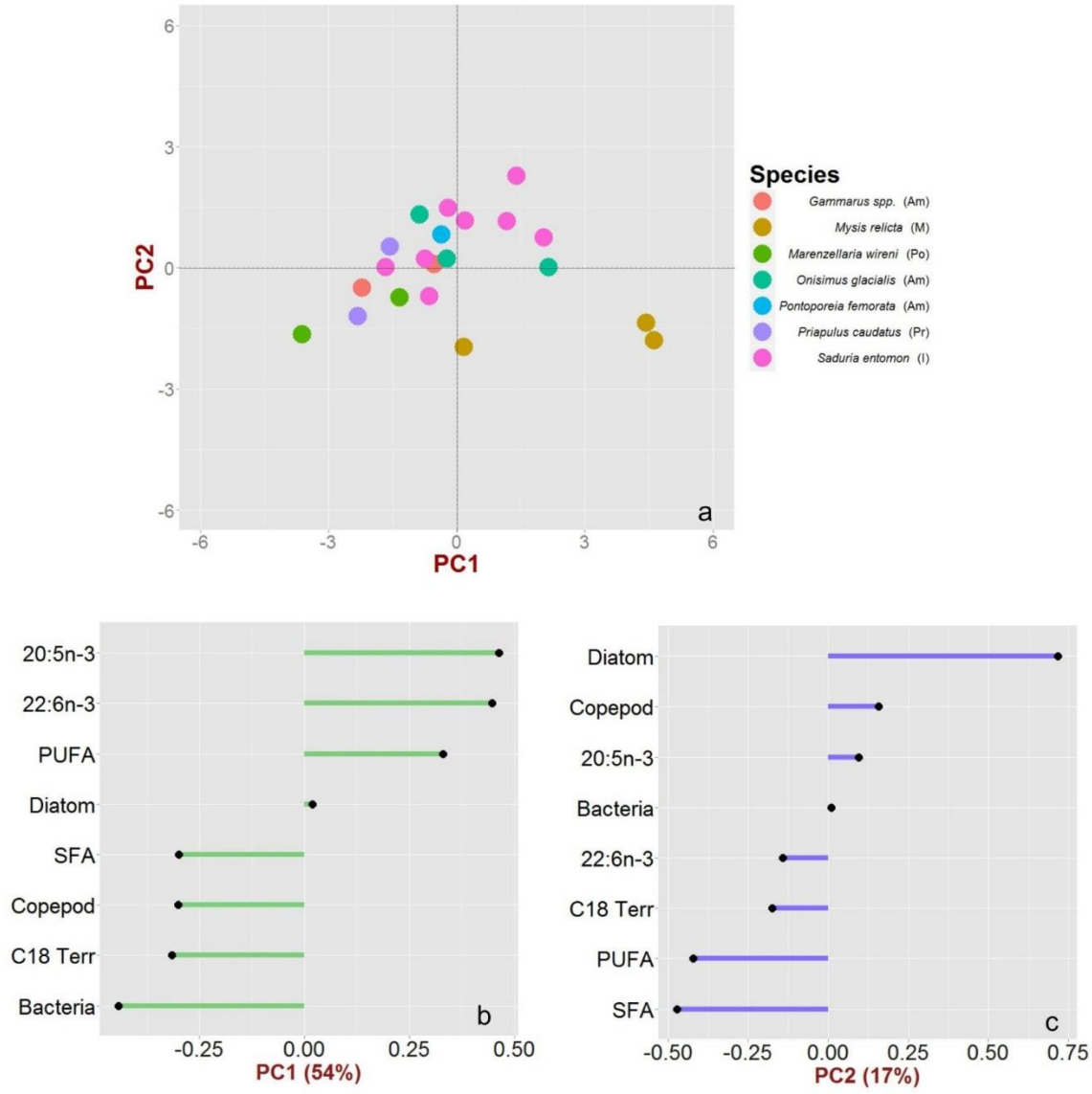

Figure 1.7. Scores (a) from the June principle component analysis for FA biomarkers in invertebrates collected from Alaskan Beaufort Sea nearshore sites and lagoons, and loadings of variables on PC1 (b) and PC2 (c). Taxa are colored based on taxonomic genus and species.

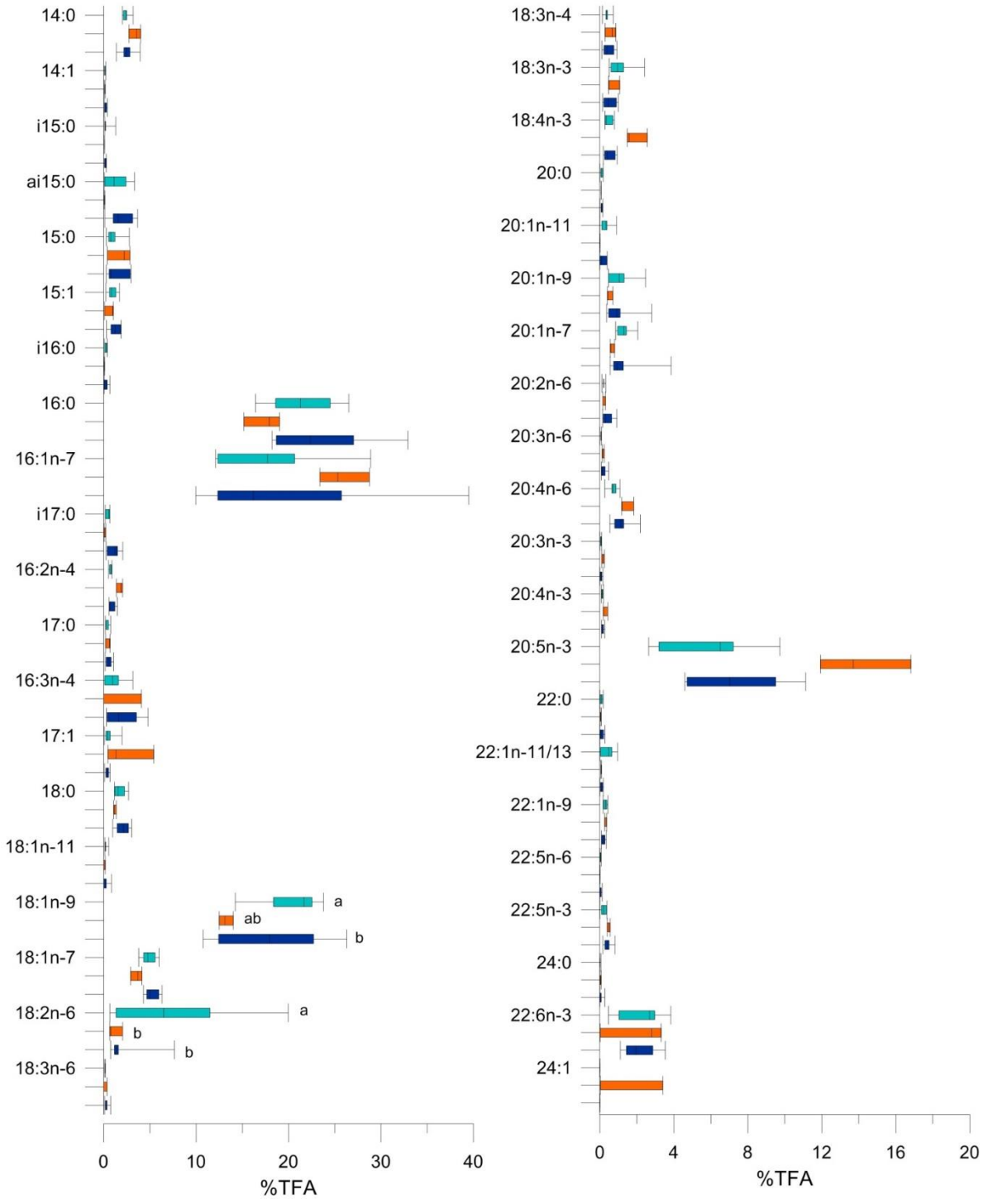

Figure 1.8 continued on next page.

Figure 1.8. Fatty acid profiles of *Onisimus glacialis* collected in April (n=6), June (n=3), and August (n=7). See Fig. 1 for definitions of box and whiskers. Seasonal values that were statistically similar or dissimilar are noted (a, b, ab), based on one-way ANOVA and pairwise comparison with an adjusted p-value.

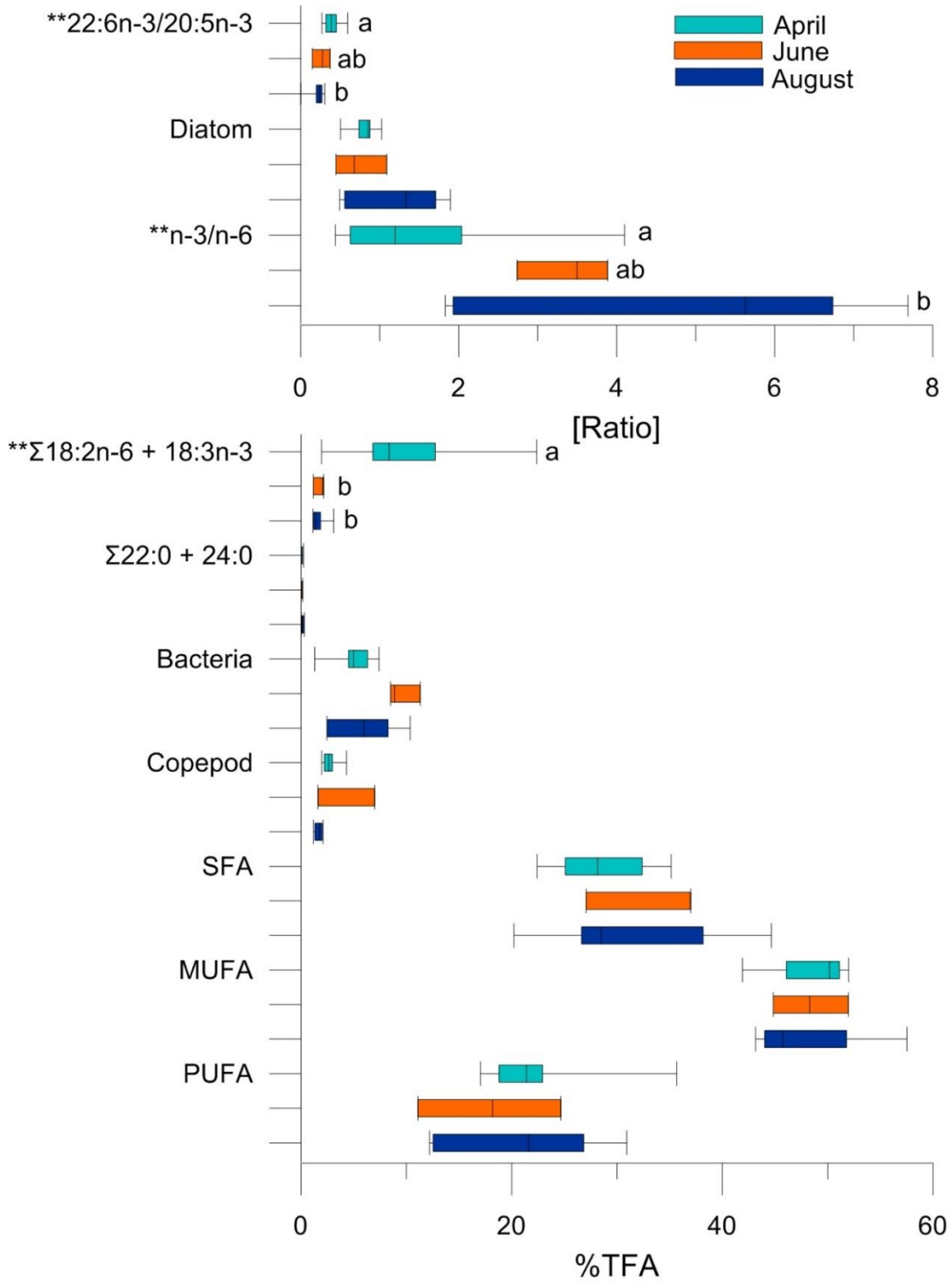

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Figure 1.9. Proportions of FA biomarkers of *Onisimus glacialis* collected in April (n=6), June (n=3), and August (n=7). See Fig. 1 for definitions of box and whiskers. **Significant difference among seasons based on ANOVA. Months that were statistically similar or dissimilar are noted (a, b, ab), based on a pairwise comparison with an adjusted p-value.

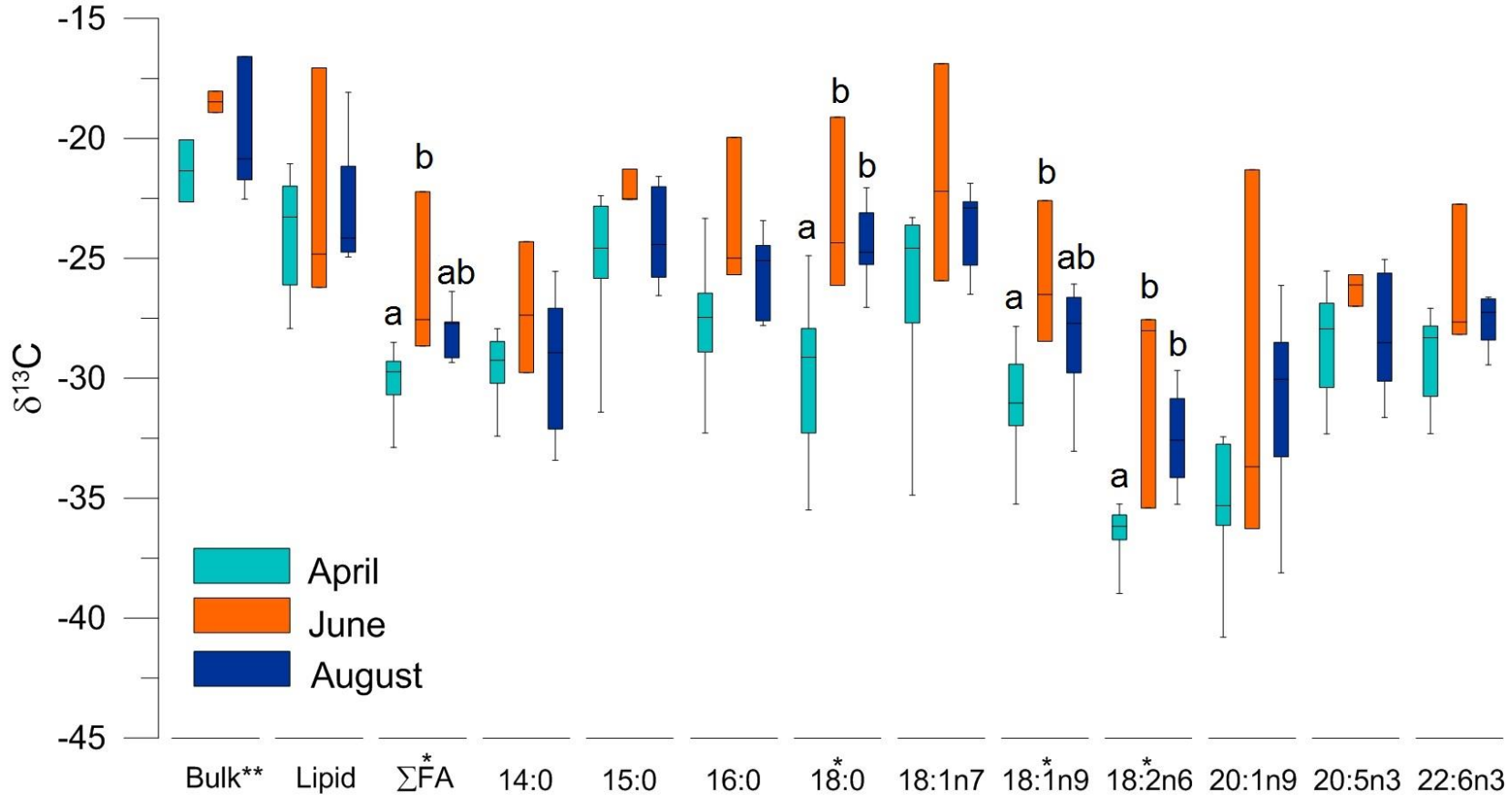

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Figure 1.10. Carbon stable isotope values of bulk material, total lipid extracts, and individual fatty acids of *Onisimus glacialis* collected in Beaufort Sea nearshore sites and lagoons in April, June, and August. See Fig. 1 for definitions of box and whiskers. The mean carbon stable isotope value of all fatty acids is denoted as ∑FA. One-way ANOVA revealed significant differences between season ($p < 0.05$), as denoted by (*). Post-hoc pairwise comparisons show months that were similar and dissimilar, noted by letters a and b. Bulk stable isotope values were collected by Harris et al. (*in prep*), and (**) notes a significant difference between April and June samples, according to Welch's t-test. Bulk isotope sample numbers are as follows: April, n=4; June, n=2; August, n=8.

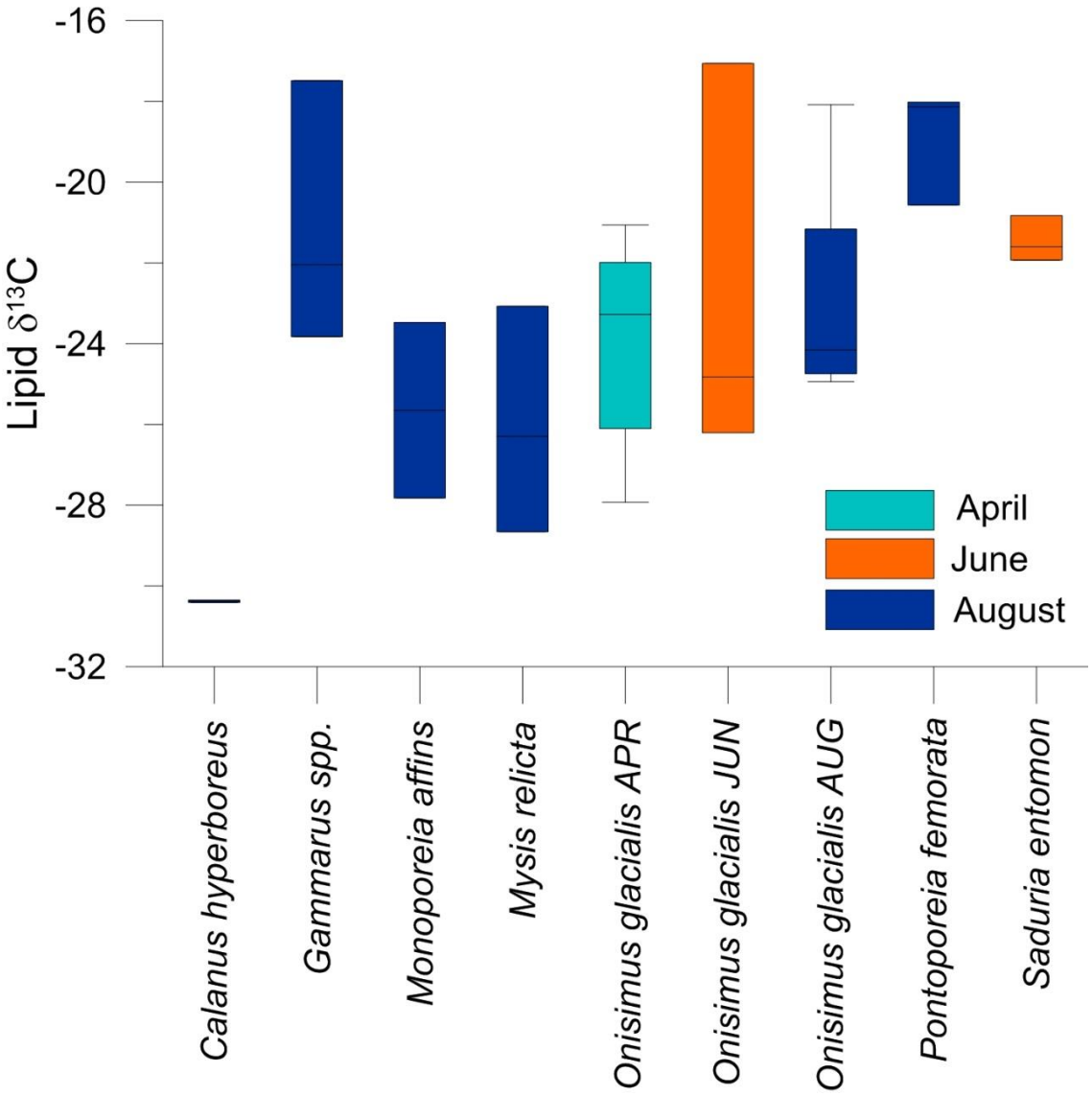

Figure 1.11. Carbon stable isotope values of total lipid extracts from a subset of invertebrates collected in Beaufort Sea nearshore sites and lagoons in April, June, and August. See Fig. 1 for definitions of box and whiskers. Sample sizes are as follows: *Calanus hyperboreus* (n=2), *Gammarus spp.* (n=3), *Monoporeia affins* (n=2), *Mysis relicta* (n=3), *Onisimus glacialis* (APR n=6; JUN n=3; AUG n=7), *Pontoporeia femorata* (n=3), *Saduria entomon* (n=3). All species, except *Calanus hyperboreus*, are peracarida crustaceans.

Chapter 2: Fatty acid biomarkers and tissue-specific turnover: validation from a controlled feeding study in a juvenile marine fish

ABSTRACT

Source-specific tracers such as fatty acids (FAs) provide a time-integrated measure of diet in aquatic food webs. To take full advantage of the information provided by FA analyses, however, assimilation patterns and incorporation rates must be constrained. In this 15-week experiment, juvenile Atlantic croaker (*Micropogonias undulatus*) were fed diets with high amounts of marine FAs (MAR), high terrestrial FAs (TER), or a mixture (MIX) of both to determine incorporation rates of terrestrial FA (18:2n-6) in liver and muscle tissues. Essential FAs (DHA, 22:6n-3 and EPA, 20:5n-3) decreased and 18:2n-6 increased in tissues of croaker fed the TER and MIX diets. Non-linear models were used to estimate incorporation rate and days to saturation of %18:2n-6 tissues. Livers incorporated 18:2n-6 faster than muscle, but the proportions of 18:2n-6 in muscles was higher than in livers. Calibration coefficients (CC) were established to determine proportions of FA deposition in tissues relative to food. Many CC were consistent amongst diet treatments, despite growth and dietary differences. The CC can be used to discern FA modification and retention within tissues, and as tools for future quantitative estimates of diet histories.

2.1 INTRODUCTION

Characterizing food web relationships, including sources and sinks of organic matter, is paramount to understanding trophic ecology and ecosystem dynamics (Pimm et al., 1991). A central motive for determining food web relationships is to develop predictive strategies for managing ecosystems in light of current and future change. A variety of tools, such as fatty acid (FA) analyses, are currently used to help define relationships between consumers and their food sources. Fatty acids are useful diet indicators because they provide a time-integrated measure of assimilated sources, unlike gut- and fecal-content analysis, and are generally passed conservatively from food source to consumer (Dalsgaard et al., 2003; Iverson, 2009). Stable isotope ratios can also be used as time-integrated indicators of diet, but they differ in their ability to distinguish among certain primary producers (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). For

example, different groups of phytoplankton (e.g. diatoms and dinoflagellates) are marked by distinct differences in fatty acid profiles, whereas their carbon stable isotope composition may be similar. Furthermore, FA biomarkers can be used to differentiate between terrestrial and aquatic primary production, help distinguish among omnivory, herbivory and carnivory, and identify bacterial contributions to diet (Napolitano, 1999; Dalsgaard et al., 2003; Iverson et al., 2004; Stevens et al., 2004). Together, measurements of FAs and stable isotopes can be used to minimize the limitations inherent in each method and thus better discern trophic relationships (Allan et al., 2010; Carreón-Palau et al., 2013; Connelly et al., 2014).

Aside from being indicators of diet-source, FAs also serve a nutritional role for marine consumers. It is widely accepted that ARA (20:4n-6), EPA (20:5n-3), and DHA (22:6n-3) are the most important long-chain polyunsaturated fatty acids (PUFAs) in invertebrates, birds, mammals and fishes, because they are required for normal somatic growth, neural development, reproduction, survival, and pigmentation (Parrish, 2009; Sargent et al., 1999). Less attention has been given to DPA (22:5n-3 and 22:5n-6), yet some suggest it may be essential to fish in early life stages (Parrish et al., 2007). These specific PUFAs are known as essential fatty acids (EFAs), because they cannot be synthesized *de novo* in sufficient quantities to meet nutritional needs and, thus, must be supplied by the diet (Bell and Wolfgang, 2010; Tocher, 2003). Availability of specific EFAs in the food web can affect fish community composition at decadal (Litzow et al., 2006) and interannual time scales (Litz et al., 2010), influencing the efficiency of carbon flow between trophic levels (Müller-Navarra et al., 2000). Therefore, identifying the trophic transfer of EFAs within a food web is important for estimating the overall health and productivity within a population and a community (Müller-Navarra et al., 2004).

The conservative nature of PUFAs and the ability to identify diet sources can also be used to address questions about habitat use and migration patterns (Copeman et al., 2009; Egeler and Williams, 2000; Watt and Ferguson, 2015). Short-chain C_{18} PUFAs (e.g. 18:2n-6 and 18:3n-3) are predominantly sourced from terrestrial and estuarine primary producers including: seagrass (Trust Hammer et al., 1998), marsh grass (Schultz and Quinn, 1973), green macroalgae (Kumari et al.,

2013), cyanobacteria (Sahu et al., 2013), mangroves and other terrestrial plants (Carreón-Palau et al., 2013). In this paper, we use the term "terrestrial FAs" to include these C_{18} PUFAs sourced from both terrestrial and near-shore producers. In contrast, marine phytoplankton and some macroalgae are predominant producers of "marine FAs" (EPA, DPA, DHA) (Dalsgaard et al., 2003; Galloway et al., 2012). Previous work has found that elevated levels of terrestrial FAs (18:2n-6 and 18:3n-3) in marine consumers indicate near-shore habitat use (Copeman et al., 2009). Therefore, we expect estuarine-dependent species to have higher levels of terrestrial FA biomarkers during periods of near-shore residency than during offshore residency.

In fishes, qualitative FA analyses have been used to describe shifts in feeding and habitat use during different life stages (St. John and Lund, 1996), but few quantitative studies exist. More recently, FAs have been used to develop a quantitative assessment of consumer diets known as quantitative fatty acid signature analysis (QFASA) (e.g. Iverson et al., 2004). This statistical technique is based on the assumption that food sources have distinct FA signatures, and that these signatures are accumulated in consumer tissues in a predicable way. By comparing the FA composition of all potential food sources to that of the predator, an estimate of diet can be obtained (Budge et al., 2006). The first step of QFASA is to perform a controlled feeding study to calculate weighting factors, known as "calibration coefficients". After feeding a known diet, saturation is reached and calibration coefficients (CCs) are calculated for each individual FA to determine the extent of FA metabolism or deposition into tissues (Iverson et al., 2004). While QFASA has been applied widely to marine mammals and birds (Bowen and Iverson, 2012; McKinney et al., 2013; Rosen and Tollit, 2012; Wang et al., 2014b), very few studies have used this approach for fishes (e.g. Budge et al., 2012; Magnone et al., 2015). Fatty acid composition in fishes may differ depending on life stage, growth, and tissue type. To address these unknown factors, controlled feeding experiments are needed.

In this study, feeding experiments were conducted using juvenile Atlantic croaker (*Micropogonias undulatus*, Linnaeus, 1766) to determine shifts in FA composition in liver and muscle tissues and to estimate the incorporation rate of terrestrial FAs. Atlantic croaker is an

important commercial and recreational estuarine-dependent fish species that is widespread in the Atlantic Ocean and Gulf of Mexico. Demersal croaker spawn offshore, producing larvae that settle into low salinity estuarine habitats dominated by vegetation (e.g. seagrass, marsh grass) and terrestrial runoff (Petrik et al., 1999; Rooker et al., 1998). Adult croaker and young-of-year occupy estuarine habitats for growth and feeding (Nye et al., 2010). To understand coastal habitat use and migration patterns of Atlantic croaker, we conducted controlled laboratory studies measuring the incorporation rates of terrestrial FAs, and the CCs of FAs after a diet switch. All fish were initially reared on the same marine FA-rich control diet. At the onset of the experiment, fish were separated into three feeding treatments, which contained differing amounts of marine FAs (EPA, DPA, DHA) and terrestrial FAs (18:2n-6 and 18:3n-3). Our main objective was to determine the incorporation rates of 18:2n-6 deposited in liver and muscle tissue. In addition, this is the only study to calculate CCs of juvenile marine fish from a subtropical region. To our knowledge, only three other studies calculate CCs of fish in controlled feeding studies (i.e. adult salmon and juvenile gadids in temperate regions, and adult flatfish in a subtropical region) (Budge et al., 2012; Copeman et al., 2013; Magnone et al., 2015, respectively). These tissue-specific coefficients can be applied to future studies for quantitative diet analyses, and provide knowledge about retention and modification of FA via elongation and desaturation processes.

2.2. MATERIALS AND METHODS

2.2.1. Fish and experimental protocol

The following experimental protocol was approved by the Institutional Animal Care and Use Committee (AUP-2013-0083) at the University of Texas at Austin. Details of larval rearing can be found in Mohan et al. 2015 (*In Review*). Briefly, eggs were collected from natural spawns of adult Atlantic croaker, sourced from local Texas-populations. Eggs were then bathed in 1-ppm formalin for 30 minutes to prevent infection and transferred to 150 L conical tanks for hatching. Hatched larvae were fed rotifers (enriched with RotiGrow Plus) that were grown on *Isochrysis galbana* algae, then the larvae were switched to artemia (enriched with Algal-Mac 3050). After

reaching juvenile size (60 days), fish were transferred to experimental tanks and fed dry pellet control diets (Table 1, Otohime EP3, Reed Mariculture, Inc.). Over the course of the experimental period, tank temperatures ranged from 23-24°C, with salinities ranging from 34-38. Upon reaching experimental size (110±10 mm total length (TL), 17±5 g), each fish was tagged with Visual Implant Alpha IV tags (Northwest Marine Technologies, Inc.) to track individual growth rates, and acclimated for 10 more days before initiating the diet switch. To estimate growth rates, an exponential growth constant (k) was calculated by fitting the data to an exponential growth model: $w_t = w_i * e^{kt}$, where w_t is the weight of fish at time t, w_i is the initial weight of the fish, and t is time (Fig. 2.1).

Fish were randomly assigned to nine 450 L tanks at densities of approximately 35 fish per tank. Diet treatments included feeding the fish one of three types of food: marine control (MAR; Otohime EP3, Reed Mariculture, Inc.), mixed (MIX; Aquafeed 4512 (FK), Cargill Animal Nutrition), and terrestrial (TER; Production 32, Rangen Connatural Products), each with a unique lipid content and FA composition (Table 2.1). The MAR diet was consider to be "marine" due to the high prevalence of EFAs from fish meal (e.g. EPA, DPA, DHA) and low levels of terrestrially-derived FAs (e.g. 18:2n-6 and 18:3n-3). The TER diet had high levels of the terrestrially-derived FA, 18:2n-6, from plant and grain by-products (q.v. manufacturer label), and lower levels of EFAs. The MIX diet had intermediate levels of both EFAs and terrestrial FAs. Duplicate samples from the food were collected at day 0 and day 32 to obtain average FA composition values (n=4). At day 0 (T0), before the diet switch, a subset of fish (n=4) was sacrificed to determine initial fish mass, and FA composition of liver and muscle tissues (Table 2.2). Then, three fish from each diet treatment were randomly sampled at day 7, 18, 32, 52, and 104. Fish were euthanized with a lethal dose of MS-222 and placed on ice. Upon dissection, each fish was patted dry and weighed to the nearest 0.01 g. The Visual Implant Alpha tag was removed and the fish identification number was noted. A fillet of dorsal white muscle tissue and liver (~100 mg per dry weight) was collected, rinsed with deionized water, weighed, and placed in vials with 2 ml of chloroform and frozen at -80°C under N_2 gas until lipid extraction, within ca. 6 months.

2.2.2. Lipid extraction and fatty acid analysis

Lipids were extracted from tissue samples in chloroform:methanol:water (2:1:0.5) following Parrish (1999), modified from Folch et al. (1957). Briefly, tissue samples were ground to a pulp using a Teflon capped metal rod, sonicated, and centrifuged in the chloroform:methanol:water mixture. The lipid layer was removed to a clean vial, and the procedure was repeated for a total of 3 times. The lipid extract was blown dry with N_2 gas and total lipid content was measured gravimetrically. Fatty acids were transformed to FA methyl esters (FAME) by derivatizing samples with BF3-methanol at 85°C for 1.5 h. FAME were quantified on a Shimadzu GC-FID with a ZB-WAX plus column (Phenomenex; 30 m, 0.53 mm id, 1.0 μm film thickness). Retention times of FA peaks of known standards (Supleco COMP 37, BAME, PUFA 1, PUFA 3) were used to identify FA peaks within samples. An internal standard (23:0) was added to each sample to quantify peaks. Fatty acids are expressed proportionally as a percentage of total identified FA. The DW of the tissues was estimated using a subset of 115 samples applied to a linear model of wet weight vs. dry weight ($y = 0.2549 x – 24.075$, $R^2 = 0.92$ for muscle; $y = 0.438x – 15.663$, $R^2 = 0.91$ for liver).

2.2.3. Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences in lipid and FA contents among diets using R Studio statistical software, where *p*-values < .05 were considered significantly different. A pairwise t-test with a Bonferroni correction was used to determine which diets had the significantly highest or lowest values. For each diet treatment, a t-test was used to compare total lipid and individual FA proportions in liver and muscle tissue at day 0 and day 104.

Uptake and incorporation of the most abundant terrestrial marker, linoleic acid (%18:2n-6), was described by a 3-parameter exponential rise to a maximum model**:** $\mathbf{Y_x = Y_0 + a[1 - e^{-bX}]}$ as applied by Copeman et al. (2013) to juvenile gadids (R Project for Statistical Computing), where **a** refers to the estimate of maximum %18:2n-6 found in tissues at saturation, **b** represents the initial slope of the FA uptake curve (%·day^{-1}), $\mathbf{Y_0}$ is the initial %18:2n-6 in the tissue at day 0, $\mathbf{Y_x}$ is the

%18:2n-6 in the tissue at day **X**, and 1/b describes the days to saturation. Proportional changes in the sum of marine fatty acids (EPA, DPA, DHA) over the 104 day experiment were fitted using a smooth-spline fit to muscle and liver tissue data (Figs. 2.2c and 2.2d).

Calibration coefficients were calculated for each individual tissue and diet treatment at day 104 by dividing the proportion of an individual FA in the croaker tissue by the average proportion of the same FA in the diet (Budge et al., 2012; Copeman et al., 2013; Iverson et al., 2004). Calibration coefficients are used to determine the extent of FA deposition in tissues, and can be used to estimate diet by accounting for FA-specific metabolism. Tissues sampled at day 104 are expected to be stabilized after the diet switch, based on other studies (e.g. Budge et al., 2011; Copeman et al., 2013).

2.3. RESULTS

2.3.1. Diet lipid content and fatty acid composition

The three manufactured fish feeds contained differing amounts of total lipid and total FAs (Table 2.1). The MAR diet had the highest lipid content (18% DW) and marine FA (DHA + EPA + DPA) proportions (25%). In contrast, the TER diet had the lowest total lipid content (7% DW), and the highest terrestrial FA (18:2n-6 + 18:3n-3) proportions (33%). The MIX diet had intermediate levels of total lipid content (13% DW), marine FAs (14%) and terrestrial FAs (17%). Ratios of n-3:n-6 followed the same pattern as total lipid content and marine FA, being highest (4.0) in MAR, intermediate (1.0) in MIX, and lowest (0.2) in TER.

2.3.2. Growth rates

Growth rates were similar between MAR (*k* 95% confidence interval (CI)=0.0085-0.012) and MIX (*k* 95% CI=0.009-0.012) treatments over the 104 day experiment, with no significant differences (Fig. 2.1). TER-fed fish had a reduced growth rate (*k* 95% CI=0.003-0.006) that was significantly different from the other two treatments, indicated by *k* with non-overlapping 95% CI.

2.3.3. Temporal changes in total lipid and FA content

At the start of the experiment (day 0), croaker livers contained 46% total lipid, but increased by day 104 to 77% in MAR livers, 56% in MIX, and 63% in TER (Table 2), although the differences between day 0 and day 104 were not significantly different among treatments. Croaker muscles contained 15% total lipid at day 0, and increased to between 26% and 32% at day 104 for all three diet treatments (Table 2.2). Only lipid content in fish muscle from the TER diet was significantly different from day 0 to 104 ($p < .01$).

The dietary effects of terrestrial FA consumption were observed in livers of TER and MIX fed fish (Table 2.2). In TER fed fish, the predominant FAs responding to the diet change were 18:1n-9 and 18:2n-6, which increased in liver tissue from day 0 to day 104 from 19% to 26% and 1% to 12%, respectively. The proportional increases in these two FAs corresponded with a decrease in the proportions of 16:0, 16:1n-7 and the marine FAs EPA and DHA (Table 2.2). The same trend for 18:1n-9 and 18:2n-6 was seen to a lesser degree in livers of fish fed the MIX diet (18:1n-9: 19% to 23% and 18:2n-6: 1% to 6% from day 0 to day 104). Livers of fish fed the MAR diet exhibited slight increases (from 1-3%) in EPA, DPA and DHA, with corresponding decreases in 16:0.

Croaker muscles exhibited a similar trend to livers within diet treatment (Table 2.2). Proportions of 18:2n-6 in muscles of TER fed fish increased from 3% to 16% from day 0 to 104. Muscles from the TER treatment also showed relative increases in 16:0, 18:0 and 18:3n-3 (by 1-2%), with proportional decreases in 14:0, EPA, DPA, and DHA (by 1-7%) (Table 2.2, Fig. 2.2c). In contrast, muscle of MIX fed fish increased in proportions of 18:0 and 18:2n-6 (by 2-6%), decreased in proportions of 14:0 (by 1%) and changed little in marine FAs over the course of the experiment (Table 2.2, Fig. 2.2c). Proportions of FAs in the control diet fish (MAR) stayed relatively constant throughout, with slight increases in DHA, 20:1n-9, and 18:1n-7 (by 2-4%), and slight decreases in terrestrial FAs and 14:0 (by 1-2%).

2.3.4. Incorporation rate of 18:2n-6

The proportion of terrestrially-derived 18:2n-6 increased over time in fish fed the MIX and TER diets, and remained relatively constant in MAR (Fig. 2.2). The "exponential rise to a maximum" model (e.g. Cober et al., 2006) was used to estimate the incorporation rate of %18:2n-6 in croaker tissues throughout the 15-week experiment (Fig. 2.2). For both muscle and liver tissues, the slope (**b**) of the line decreased until reaching a saturation maximum (**a**). Model parameters (Table 2.3) show a higher uptake rate in MIX-fed fish (0.09%·day^{-1} for liver; 0.02%·day^{-1} for muscle) compared to TER-fed fish (0.02%·day^{-1} for liver and 0.01%·day^{-1} for muscle). Correspondingly, we estimated that saturation of %18:2n-6 (1/b) in tissues occurred at approximately 81 days for TER-fed fish muscle and 46 days for liver. In contrast, incorporation of %18:2n-6 for MIX-fed fish was faster with saturation at approximately 44 days for muscle and 11 days for liver tissue. The estimated maxima %18:2n-6 in MIX muscle (6.4%±0.6 S.E.) and liver (3.3±0.4) were lower than empirical values collected at day 104 (9.0±0.0% for muscle; 5.6±0.9% for liver). But, empirical values for TER muscle and liver collected at day 104 were within two standard errors of the model estimates.

2.3.5. Calibration coefficients

Tissue-specific CCs were determined for day 104, and were generally consistent amongst the three diet treatments (Fig. 2.3). In particular, average CCs for 14:0, 16:0, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, ARA and EPA were the most consistent and had the least amount of variance (SE < 0.05) (Table 2.4). Although tissues from each diet treatment had variable proportions of these eight FAs, CCs were constant, indicating the reliability of these FA as tracers for diet-source studies. Mean CCs for all measured FA >0.05% in tissues are included in supplementary material (Table B1).

2.4. DISCUSSION

The aim of this study was to evaluate the application of FA biomarkers as a source-specific tracer of diet within a migratory marine fish. To do so, FA profiles of juvenile Atlantic croaker

and incorporation rates of a near-shore "terrestrial" FA (18:2n-6) within tissues were determined over the course of 15 weeks following a diet switch. Calibration coefficients were calculated to quantify the extent of FA deposition in tissues, and to identify potential modification of FA via elongation or desaturation. The results indicate that FA are susceptible to rapid compositional changes within fish tissues, and the magnitude of change is dependent on the dietary FA composition, days of feeding, tissue type, FA modification, and growth.

2.4.1. Influence of tissue type

When applying FA biomarkers to fish, an important consideration is choosing an appropriate tissue type to analyze. Fatty fishes (e.g. salmonids, herring, sea bream) accumulate lipids and FAs primarily in muscle tissues, whereas lean fishes (e.g. cod, haddock, flounder) store relatively more lipids and FAs in liver tissues (Ando et al., 1993; Budge et al., 2011; Copeman and Parrish, 2004; Guil-Guerrero et al., 2011; Nanton et al., 2007; Zeng et al., 2010). In lean fishes, muscles generally have higher amounts of polar lipids, like phospholipids (PL), while livers have higher amounts of neutral lipids (NL), like triacylglycerols (TAG), which are used for energy storage (Copeman and Parrish, 2004). NL are influenced by dietary changes in FA composition more quickly than polar lipids (Castledine and Buckley, 1980; Koussoroplis et al., 2010). Polar PL are highly specialized to form the structure of cellular membranes and are generally conserved in tissues regardless of diet (Böhm et al., 2014). Neutral TAG are deposited during fattening and stored in the liver and adipose tissue. The FAs in TAG are deposited into tissues relatively unmodified from the diet, making TAG reliable dietary tracers (Iverson, 2009). Therefore, livers of lean fish with high amounts of TAG and low amounts of PL would be the most appropriate tissue to analyze for studies monitoring rapid changes in diet. Atlantic croaker are lean fish with higher total lipid content in livers (~65%) compared to muscle (~29%), according to our data of juveniles. Field studies of Atlantic croaker reported lipid content in muscles to be approximately 13-32% per dry weight, with notable increases in summer months (Gallagher et al., 1991). Thus,

our experimental fish were within the range expected for wild Atlantic croaker, and the results are applicable to field studies in subtropical systems.

Although we did not separate FAs into neutral and polar lipid classes, we can reasonably assume that the metabolically active livers predominantly contained FAs derived from NL, and muscles contained more FAs derived from cell membrane polar PL. Polar lipids generally contain higher amounts of PUFAs than neutral lipids do (Böhm et al., 2014). In our study, total %PUFAs was higher in croaker muscles (~33%) compared to livers (~19%), signaling a predominance of polar PL in muscle tissue. As a result, the neutral-TAG-rich livers in our experimental fish responded more quickly to the diet switch by incorporating 18:2n-6 at a faster rate than muscles. Livers may be the best indicators of rapid diet change, but muscles may provide a longer time-integrated measure of diet. By analyzing lipid pools from muscles and livers separately, then comparing the proportions of FAs in a ratio of liver to muscle, temporal trends may be revealed that provide information about tissue equilibration time that can be used to understand habitat use and feeding ecology. Copeman et al. (2013), measured the ratio of 18:3n-3 in liver:muscle of juvenile gadids over time, and found that the greatest differences were observed after 4 weeks of feeding a terrestrial diet, and equilibrium between tissues was reached after 6 weeks. We calculated the ratio of 18:2n-6 in liver:muscle of our experimental Atlantic croaker and found a similar pattern of maximal differences occurring after 2 weeks in MIX-fed fish and 4 weeks in TER-fed fish (Fig. B1; Appendix). In both MIX and TER treatments, tissue equilibrium was reached after 7 weeks. By day 104, at equilibrium, all three diets had ratios within the range of 0.6 – 0.8. Therefore, in field studies of Atlantic croaker, high ratios (≥1) may indicate a new diet and near shore residency of approximately 4 weeks.

When switched from the MAR diet to MIX and TER diets, juvenile Atlantic croaker incorporated 18:2n-6 at a rate of 0.01 to 0.09%·day^{-1} (Table 2.3), with higher incorporation rates in livers than muscles. Muscles of TER-fed fish had the lowest incorporation rate (0.01%·day^{-1}), which may reflect low growth rates due to the nutritionally-poor TER diet. Despite the variable observed growth rates in different diet treatments, our incorporation rates are comparable with

other lab and field studies of juvenile marine fish. Copeman et al. (2013) reported an incorporation rate of 18:2n-6 at 0.02 and 0.12%·day^{-1} in experimental temperate juvenile gadid muscle and liver tissue, respectively, indicating that our calibrated uptake rates may be reasonably applied to other juvenile fishes, regardless of latitude (i.e. subtropics, temperate zones, etc.). How these patterns in lean juvenile fish compare with fatty juvenile fish still needs to be determined. Currently, there are no known field studies reporting levels of terrestrial FAs in juvenile Atlantic croaker transitioning from offshore to estuarine habitats. We encourage future field studies of juvenile Atlantic croaker to focus on sampling across seasons with regard to applying these calculated incorporation rates of terrestrial FA to estimate the duration of feeding in a near shore habitat. Incorporation rates are most useful when applied in combination with other indicators such as ratios of %18:2n-6 in liver:muscle (Fig. B; Appendix), calibration coefficients and stable isotope values (e.g. Mohan et al. *In Review*).

Fatty acid biomarkers may be able to detect source-specific dietary changes more quickly than bulk stable isotopes. The exponential rise to maximum model used in our study estimated the days (1/b) it took to reach saturation of %18:2n-6 in tissues. For MIX and TER diets, time estimates were faster for livers (11 d and 47 d, respectively) compared to muscles (44 d and 81 d, respectively). Mohan et al. (*In Review*) conducted a companion study investigating the turnover of bulk stable isotopes in the same experimental Atlantic croaker. Growth and time-based non-linear models (e.g. Fry and Arnold, 1982; Hesslein et al., 1993) estimated 95% carbon turnover ($t_{95\%}$) to occur in livers at approximately 216 d and 60 d for MIX and TER, respectively. Carbon turnover ($t_{95\%}$) in muscles was estimated at approximately 129 d and 343 d for MIX and TER, respectively. All of the bulk carbon turnover estimates had a relatively high range of error. In comparison, %18:2n-6 reached saturation in tissues faster than bulk carbon isotope turnover. Notably, %18:2n-6 and bulk carbon reached saturation faster in MIX fish compared to TER fish, except in the case of TER liver, which reached carbon isotope saturation faster than MIX liver. The rapid change in TER liver carbon isotopes was attributed to metabolism-driven turnover, compared to growth-driven turnover in all other treatments and tissues (Mohan et al. *In Review*). While total FAs only

represent a small portion of bulk carbon (up to 16% in our study), it is possible that FA modification of certain excess FAs (18:2n-6) may elucidate the deviation from growth-driven turnover to metabolism-driven turnover in TER livers. In this way, FA biomarkers are able to detect rapid dietary changes and disentangle the complex mechanisms driving bulk stable isotope turnover.

2.4.2. Estimating diet contribution with calibration coefficients

Although each diet had distinctive amounts of 18:2n-6 (ranging from 4% to 31%), resulting in variable proportions of 18:2n-6 within croaker tissues (ranging from 1% to 16%), similar CCs for this terrestrial marker at the end of the experiment (Table 2.4) support the use of 18:2n-6 as a reliable tracer for diet-source studies, and may potentially be applied to past and future datasets. For example, applying our muscle CC (0.59) to previous studies of whole-bodied adult Atlantic croaker ($\%18{:}2n\text{-}6_{body}$ = 0.51 to 2.81%; e.g. Recks and Seaborn, 2008) yields an estimated dietary source containing 0.9% to 4.8% 18:2n-6. Based on the diets used in our study, 18:2n-6 dietary values greater than 5% indicate a strong influence of terrestrial FAs. However, given the differences in CC values between muscle and liver tissues reported herein, application of experimentally validated tissue-specific CC values to whole organisms should be done with caution as FA profiles likely reflect metabolism and deposition that is tissue-specific. It is also important to note that there may be differences in CCs between juveniles and adults. Currently, there are no known studies reporting FA compositions of juvenile Atlantic croaker, but future work with wild-caught fish could rely on established CCs to calculate the proportion of 18:2n-6 and other FAs derived from the diet.

Aside from 18:2n-6, eight other FAs had reliable CCs across all diet treatments, with a SE <0.05 in either tissue (Table 2.4). Notably, another "terrestrial" FA, 18:3n-3, had well constrained CCs at 0.40 and 0.51 for liver and muscle, respectively. Using CCs of 18:3n-3 in combination with 18:2n-6 would strengthen the diet estimates of terrestrial FA sources. Essential fatty acids EPA and ARA had consistent CCs for livers and muscles, showing that EPA and ARA are valid

biomarkers for diet studies. EPA in particular can be used with the ratio of 16:1n-7/16:0 to indicate a diet source derived from diatoms (Dalsgaard et al., 2003). Likewise, CCs of 16:0 were well constrained within the croaker tissues. The dinoflagellate marker (18:4n-3) was also incorporated into fish tissues with consistent CCs. Overall the six CCs of FA mentioned thus far have direct applications for tracing diet sources within food webs. The remaining FAs with consistent CC are: 14:0, 18:0, and 18:1n-9. These are dominant FAs within animal tissues and some are expected to have contributions from endogenous sources (Iverson et al., 2004).

Other essential fatty acids (e.g. DHA and n-3 DPA) had CCs that differed widely in each treatment, making them less useful for assessments of diet contribution, despite their wide use in quantitative models (e.g. QFASA model; Iverson et al., 2004). The higher CCs for DHA and DPA observed in the TER-fed fish most likely reflect enhanced retention of EFAs, possibly retained from the pre-experimental MAR diet, due to nutritional requirements. The CCs reported by Copeman et al. (2013) for juvenile gadids were similar to our results, with consistent coefficients for 18:2n-6 and 18:3n-3 across treatments and tissues. Gadids fed a high terrestrial FA diet also exhibited higher coefficients for potential elongation products like 20:2n-6 and 20:3n-3, as seen in our experiment. Some striking similarities are apparent between CCs of juvenile Atlantic croaker and juvenile gadids. Notably, between species, many CCs for short-chain FA were ≤ 1, specifically CCs of terrestrial FAs were all ~0.5. Overall, the nine CCs validated in this feeding study can be applied with confidence in future quantitative studies of diet, and potentially in studies using compound-specific stable isotopes.

2.5. CONCLUSIONS

This study demonstrated the utility of FA biomarkers for estimating diet contribution in juvenile Atlantic croaker. There is great potential that results from this study can be expanded to field-based studies of Atlantic croaker to investigate diet, health, and habitat use. Furthermore, research programs focusing on other fish species can use the results presented here as a framework for designing and implementing controlled feeding experiments using FA biomarkers.

While species-specific and tissue-specific feeding experiments are ideal for quantitative diet analysis, this approach may be impractical for broad food web studies. Instead, development of a database of CCs for a range of taxonomic groups would facilitate FA applications in trophic studies more generally. Many CCs validated in our study were comparable, if not identical to those found in other studies (i.e. Copeman et al., 2013). To use FA biomarkers for quantitative diet contributions, a number of factors need to be addressed including: seasonality, ontogenetic state of the study organism, growth rate, incorporation rate, CCs and influence of mixed diets. Fatty acids are powerful tools for evaluating food web relationships, health, and habitat use. Yet, conclusions drawn from FA analyses are strengthened through a multi-proxy approach including stable isotopes, gut contents, and otolith microchemistry.

Table 2.1. Lipid and selected fatty acid (FA) composition (≥1% total FA) of experimental diets fed to juvenile Atlantic croaker. (n = 4, mean ± SE)

	MARINE	MIXED	TERRESTRIAL
Total lipid (% DW)*	**18.0** ± 1.1	12.9 ± 0.5	7.1 ± 0.4
Fatty acid (% total FA)			
14:0*	4.3 ± 0.3	4.7 ± 0.2	2.0 ± 0.2
16:0	21.5 ± 0.6	21.8 ± 0.2	21.3 ± 0.2
16:1n-7*	5.1 ± 0.3	5.7 ± 0.1	3.3 ± 0.2
18:0*	5.3 ± 0.2	4.8 ± 0.1	5.7 ± 0.2
18:1n-9*	13.9 ± 0.8	16.8 ± 0.4	19.0 ± 1.0
18:1n-7	4.0 ± 0.7	3.6 ± 0.4	5.6 ± 0.9
18:2n-6*	4.3 ± 0.0	15.3 ± 1.0	**30.6** ± 1.3
18:3n-3*	0.9 ± 0.0	1.7 ± 0.1	**2.3** ± 0.1
18:4n-3*	1.3 ± 0.1	1.1 ± 0.1	0.5 ± 0.1
20:1n-9*	3.2 ± 0.2	1.3 ± 0.1	0.8 ± 0.1
20:4n-6* (ARA)	1.4 ± 0.1	1.3 ± 0.0	0.5 ± 0.0
20:5n-3* (EPA)	**9.8** ± 0.2	6.7 ± 0.4	2.7 ± 0.3
22:5n-3* (DPA)	1.3 ± 0.1	1.1 ± 0.1	0.4 ± 0.1
22:6n-3* (DHA)	**14.1** ± 0.5	6.3 ± 0.4	1.7 ± 0.2
∑SFA*[a]	33.5 ± 0.4	33.5 ± 0.2	30.4 ± 0.5
∑MUFA[b]	29.4 ± 0.6	29.1 ± 0.1	29.3 ± 0.5
∑PUFA*[c]	37.2 ± 0.2	37.4 ± 0.2	**40.3** ± 0.5
n-3:n-6*	**4.0** ± 0.0	1.0 ± 0.1	0.2 ± 0.0
EPA:ARA	7.1 ± 0.1	5.2 ± 0.5	5.9 ± 0.6
DHA:EPA*	**1.4** ± 0.0	0.9 ± 0.0	0.6 ± 0.0
∑Terrestrial*[d]	5.2 ± 0.0	17.0 ± 1.1	**32.9** ± 1.4
∑Marine*[e]	**25.3** ± 0.6	14.2 ± 0.9	4.7 ± 0.5

Dry weight (DW), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), arachidonic acid 20:4n-6 (ARA), eicosapentaenoic acid 20:5n-3 (EPA), docosapentaenoic acid 22:5n-3 (DPA), docosahexaenoic acid 22:5n-3 (DHA).
* Significant difference among diets based on ANOVA ($p < .05$). Only values that were significantly higher (bold) or lower (underline) than values from both of the other two diets are identified, based on a pairwise comparison with an adjusted p-value.
[a] Sum of SFA, which also includes *i*15:0, *ai*15:0, 15:0, *i*16:0, *i*17:0, *ai*17:0, 17:0, and 20:0 at < 1% each.
[b] Sum of MUFA, which also includes: 14:1, 15:1, 17:1, and 22:1n11.13 at < 1% each.
[c] Sum of PUFA, which also includes: 16:2n4, 16:3n4, 16:4n1, 18:3n6, 18:3n4, 20:2n6, 20:3n6, 20:3n3, 20:4n3, 22:5n6 at < 1% each.
[d] Terrestrial and near-shore fatty acids: 18:2n-6 and 18:3n-3.
[e] Marine fatty acids: 20:5n-3 (EPA), 22:5n-3 (DPA) and (22:6n-3) DHA.

Table 2.2. Total lipid (% dry weight (DW)), total fatty acids (FA), and selected fatty acid (≥1% total FA in at least one diet treatment) for liver and muscle of juvenile Atlantic croaker fed experimental diets for 104 days. At the start of the experiment (day 0), croaker had been feeding on control marine diet (MAR) diet for >4 months. By day 104, croaker had been feeding on either MAR, mixed (MIX), or terrestrial (TER) diet for 104 days. Values are mean ± SE, n = 4 fish at day 0, and n = 3 fish at day 104 for each diet.

Table 2.2.

	Liver Day 0	MAR Liver Day 104	MIX Liver Day 104	TER Liver Day 104	Muscle Day 0	MAR Muscle Day 104	MIX Muscle Day 104	TER Muscle Day 104
Total lipid (%DW)	45.7 ± 8.4	76.7 ± 9.2	56.5 ± 0.6	63.0 ± 8.0	14.9 ± 2.4	29.7 ± 7.7	26.3 ± 3.7	*32.0 ± 3.2
% total FA								
14:0	1.8 ± 0.3	1.9 ± 0.1	1.5 ± 0.3	1.3 ± 0.1	3.5 ± 0.3	*2.1 ± 0.2	*1.8 ± 0.1	*1.7 ± 0.1
16:0	35.5 ± 0.6	*25.8 ± 0.8	*27.9 ± 0.5	*28.1 ± 0.6	24.4 ± 0.6	24.2 ± 0.1	24.7 ± 0.3	25.3 ± 0.5
16:1n-7	14.8 ± 0.6	14.7 ± 0.5	15.9 ± 1.0	*11.4 ± 0.5	9.0 ± 0.5	7.3 ± 1.0	8.4 ± 0.1	8.1 ± 0.4
18:0	5.5 ± 0.3	4.7 ± 0.3	6.3 ± 0.9	8.0 ± 1.3	4.3 ± 0.2	5.3 ± 0.4	*5.6 ± 0.1	*6.1 ± 0.1
18:1n-9	18.5 ± 0.8	19.9 ± 0.7	*22.9 ± 1.6	*25.7 ± 0.9	18.3 ± 0.9	14.9 ± 1.4	16.8 ± 0.5	19.6 ± 1.2
18:1n-7	3.1 ± 0.3	*4.6 ± 0.0	4.0 ± 0.3	2.9 ± 0.2	3.2 ± 0.4	*5.2 ± 0.1	4.4 ± 0.3	5.3 ± 0.7
18:2n-6	1.4 ± 0.2	*2.4 ± 0.2	*5.6 ± 0.9	***12.4** ± 1.5	3.1 ± 0.1	2.9 ± 0.0	*9.0 ± 0.0	***16.0** ± 0.5
18:3n-3	0.3 ± 0.0	*0.5 ± 0.1	0.5 ± 0.1	*0.7 ± 0.1	0.7 ± 0.0	*0.6 ± 0.0	0.8 ± 0.0	***1.0** ± 0.0
20:1n-9	1.4 ± 0.4	***2.7** ± 0.0	1.1 ± 0.1	1.2 ± 0.0	0.9 ± 0.4	***2.8** ± 0.2	1.3 ± 0.1	1.5 ± 0.1
20:5n-3 (EPA)	3.4 ± 0.4	***3.4** ± 0.2	2.1 ± 0.3	*1.1 ± 0.2	6.6 ± 0.4	6.3 ± 0.2	5.6 ± 0.3	*3.1 ± 0.4
22:5n-3 (DPA)	0.9 ± 0.0	*2.1 ± 0.3	1.3 ± 0.4	0.6 ± 0.1	2.3 ± 0.1	2.5 ± 0.0	2.3 ± 0.0	*1.1 ± 0.1
22:6n-3 (DHA)	6.7 ± 0.9	8.8 ± 1.0	5.4 ± 0.9	*3.3 ± 0.6	12.8 ± 1.0	16.5 ± 2.3	12.1 ± 0.5	*6.4 ± 1.2
∑SFA[a]	44.3 ± 0.7	*34.1 ± 0.9	*37.0 ± 1.0	*38.3 ± 2.0	34.1 ± 0.7	33.1 ± 0.2	33.3 ± 0.5	34.1 ± 0.6
∑MUFA[b]	39.1 ± 1.3	*43.5 ± 1.0	44.4 ± 1.9	41.6 ± 1.6	34.1 ± 1.2	32.6 ± 3.2	32.3 ± 0.6	35.6 ± 1.2
∑PUFA[c]	16.6 ± 1.7	22.3 ± 1.8	18.6 ± 2.7	20.1 ± 2.3	31.8 ± 1.7	34.2 ± 3.1	34.4 ± 1.1	30.4 ± 1.8
n-3:n-6	3.9 ± 0.3	**3.8** ± 0.2	*1.3 ± 0.1	*0.4 ± 0.0	4.5 ± 0.1	**4.7** ± 0.2	*1.8 ± 0.1	*0.7 ± 0.1
∑Terrestrial[d]	1.7 ± 0.2	*2.9 ± 0.3	*6.2 ± 1.0	***13.0** ± 1.5	3.9 ± 0.1	*3.4 ± 0.1	*9.8 ± 0.1	***17.0** ± 0.6
∑Marine[e]	11.0 ± 1.4	**14.2** ± 1.4	8.8 ± 1.4	*5.0 ± 0.9	21.7 ± 1.2	25.4 ± 2.5	20.0 ± 0.8	*10.6 ± 1.7

* Significant difference ($p < .05$) between total lipid or individual fatty acid proportions in liver or muscle from day 0 to day 104 based on results of a pairwise t-test for each diet treatment. Values for a diet treatment at day 104 that were significantly higher (bold) or lower (underline) than values from both of the other two diet treatments are identified, based on a Bonferroni pairwise comparison with an adjusted p-value.

[a] Sum of saturated fatty acids (SFA), which also includes *i*15:0, *ai*15:0, 15:0, *i*16:0, *i*17:0, *ai*17:0, 17:0, and 20:0 at < 1% each.

[b] Sum of monounsaturated fatty acids (MUFA), which also includes: 14:1, 15:1, 17:1, and 22:1n11.13 at < 1% each.

[c] Sum of polyunsaturated fatty acids (PUFA), which also includes: 16:2n4, 16:3n4, 16:4n1, 18:3n6, 18:3n4, 20:2n6, 20:3n6, 20:3n3, 20:4n3, 22:5n6 at < 1% each.

[d] Terrestrial and near-shore fatty acids: 18:2n-6 and 18:3n-3.

[e] Marine fatty acids: 20:5n-3 (EPA), 22:5n-3 (DPA) and (22:6n-3) DHA.

Table 2.3. Maximum rise model parameters (± standard error) describing the uptake of linoleic acid (%18:2n-6) and days until reaching a saturation maximum (1/b) in muscle and liver of juvenile Atlantic croaker fed either the terrestrial (TER) or mixed (MIX) diet for 104 days. The model uses the equation $\mathbf{Y_x = Y_0 + a(1 - e^{-bX})}$, where **a** refers to the estimate of maximum %18:2n-6 found in tissues at saturation, **b** represents the initial slope of the FA uptake curve (%·day^{-1}), $\mathbf{Y_0}$ is the initial %18:2n-6 in the tissue at day 0, and **X** is the number of days.

	% 18:2n6			
Treatment	**Max % FA**	**% FA·day^{-1}**	**Days**	**R^2**
	a	**b**	**1 / b**	
Muscle-TER	18.70 ± 5.07	0.01 ± 0.01	81.2	0.88
Muscle-MIX	6.43 ± 0.58	0.02 ± 0.00	44.1	0.94
Liver-TER	12.11 ± 1.56	0.02 ± 0.01	46.5	0.90
Liver-MIX	3.25 ± 0.42	0.09 ± 0.04	11.2	0.60

Table 2.4. Mean (± standard error) fatty acid (FA) calibration coefficients (CC; $\%FA_{tissue} \div \%FA_{diet}$; mean ± SE) for muscle and liver tissue of juvenile Atlantic croaker from all three diet treatments (n = 9) after 104 days of feeding. Only CC for selected fatty acids are shown (FA >1% and CC standard error of < 0.05 in either tissue type). Fatty acids with a SE near 0 have the least amount of variation among diet treatments.

Calibration Coefficients				
Fatty acid	Liver	SE	Muscle	SE
14:0	0.46	0.05	0.57	0.07
16:0	1.26	0.02	1.15	0.01
18:0	1.19	0.12	1.07	0.04
18:1n-9	1.38	0.04	1.04	0.04
18:2n-6	0.44	0.04	0.59	0.02
18:3n-3	0.40	0.06	0.51	0.03
18:4n-3	0.35	0.05	0.50	0.04
20:4n-6	0.64	0.05	1.27	0.13
20:5n-3	0.35	0.03	0.88	0.09

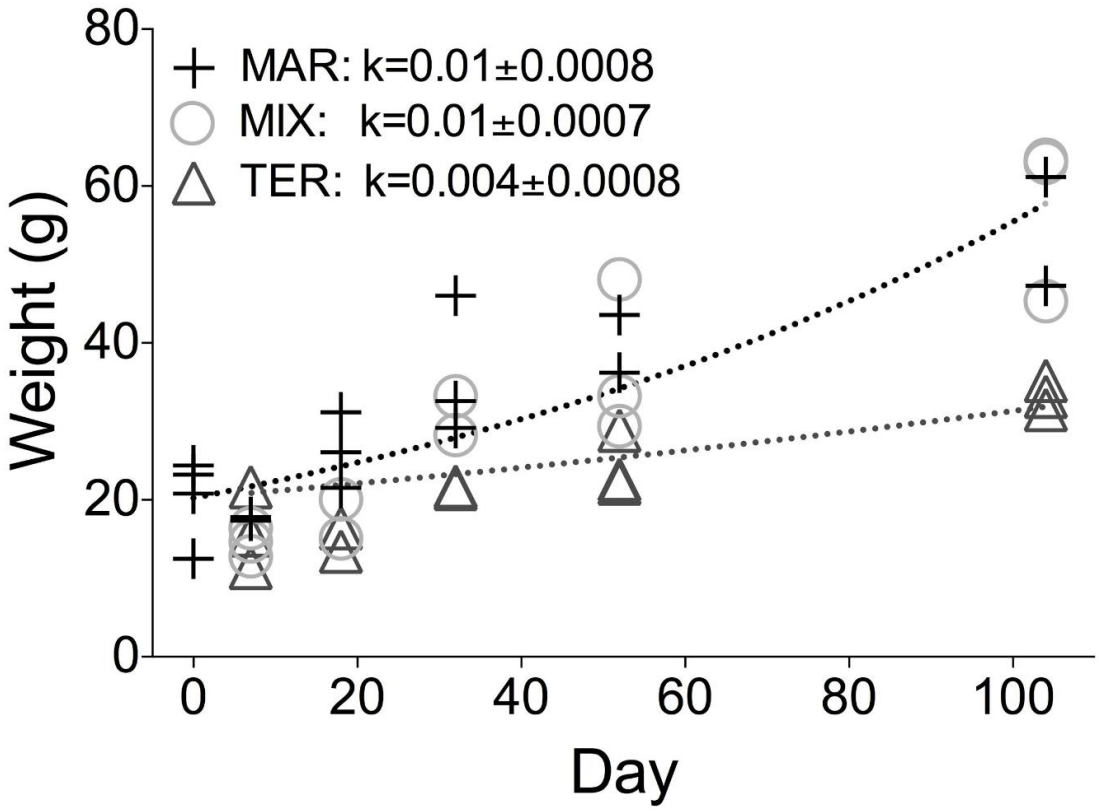

Figure 2.1. Exponential curve fits for weight gain (g) over time used to estimate the exponential growth constant *k* (± 95% confidence interval) of juvenile Atlantic croaker fed marine (MAR), mixed (MIX), or terrestrial (TER) diets for 104 days. Note that growth curves overlap for MAR and MIX.

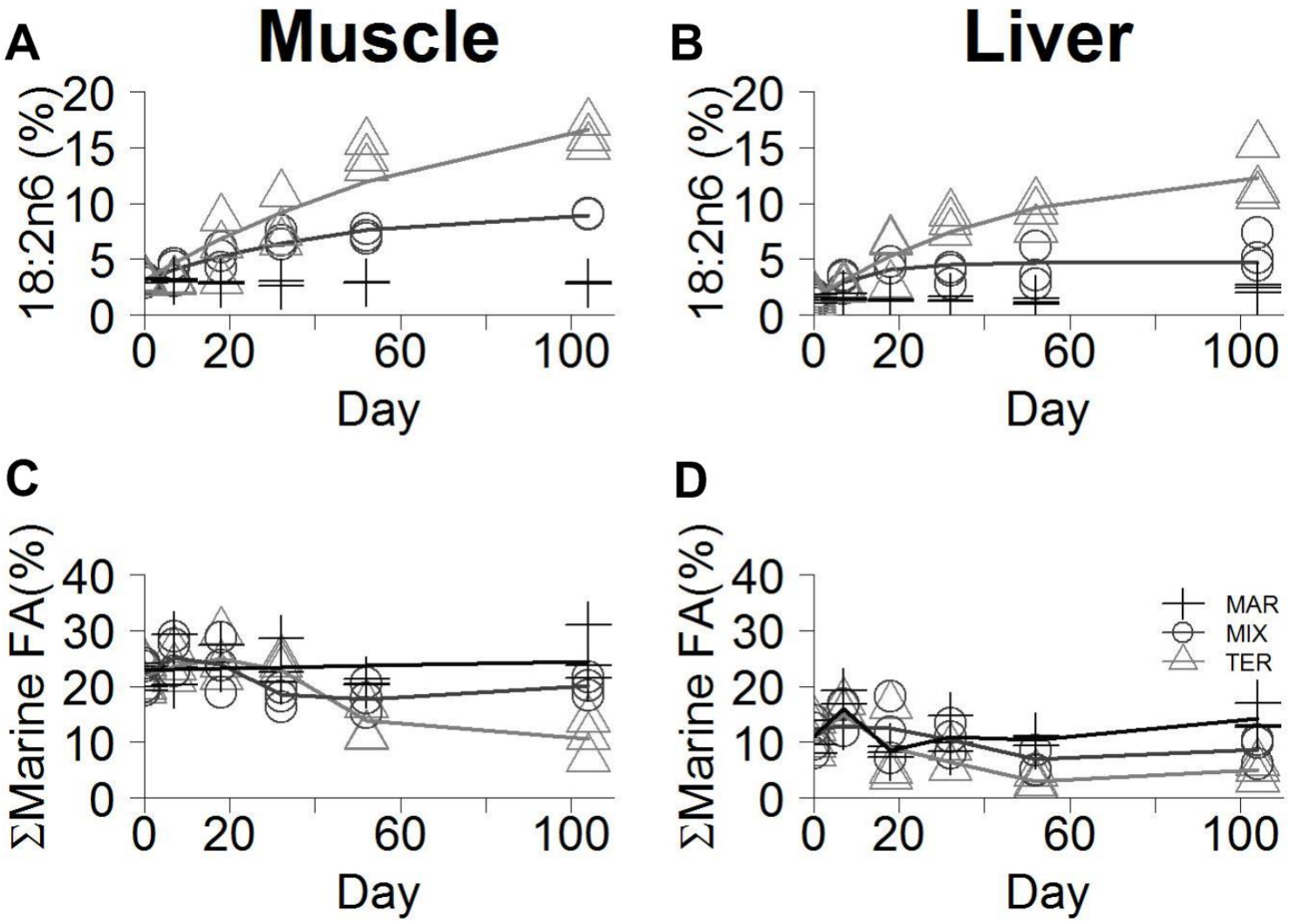

Figure 2.2. Continued next page.

Figure 2.2. Changes in proportion of 18:2n-6 in juvenile Atlantic croaker muscle (A) and liver (B) while feeding on the terrestrial (TER, triangle), mixed (MIX, circle), or marine (MAR, cross) diet for 104 days. Data for TER and MIX (%18:2n-6) was fitted to an exponential rise to a maximum curve, $Y_x = Y_0 + a(1 - e^{-bX})$. Proportional changes in the sum of n-3 marine fatty acids (EPA: 20:5n-3, DPA: 22:5n-3, and DHA: 22:6n-3) were fitted using a smooth-spline in croaker muscle (C) and liver (D) for all three diets. Symbols represent the %fatty acid (FA) in each individual fish and diet treatment.

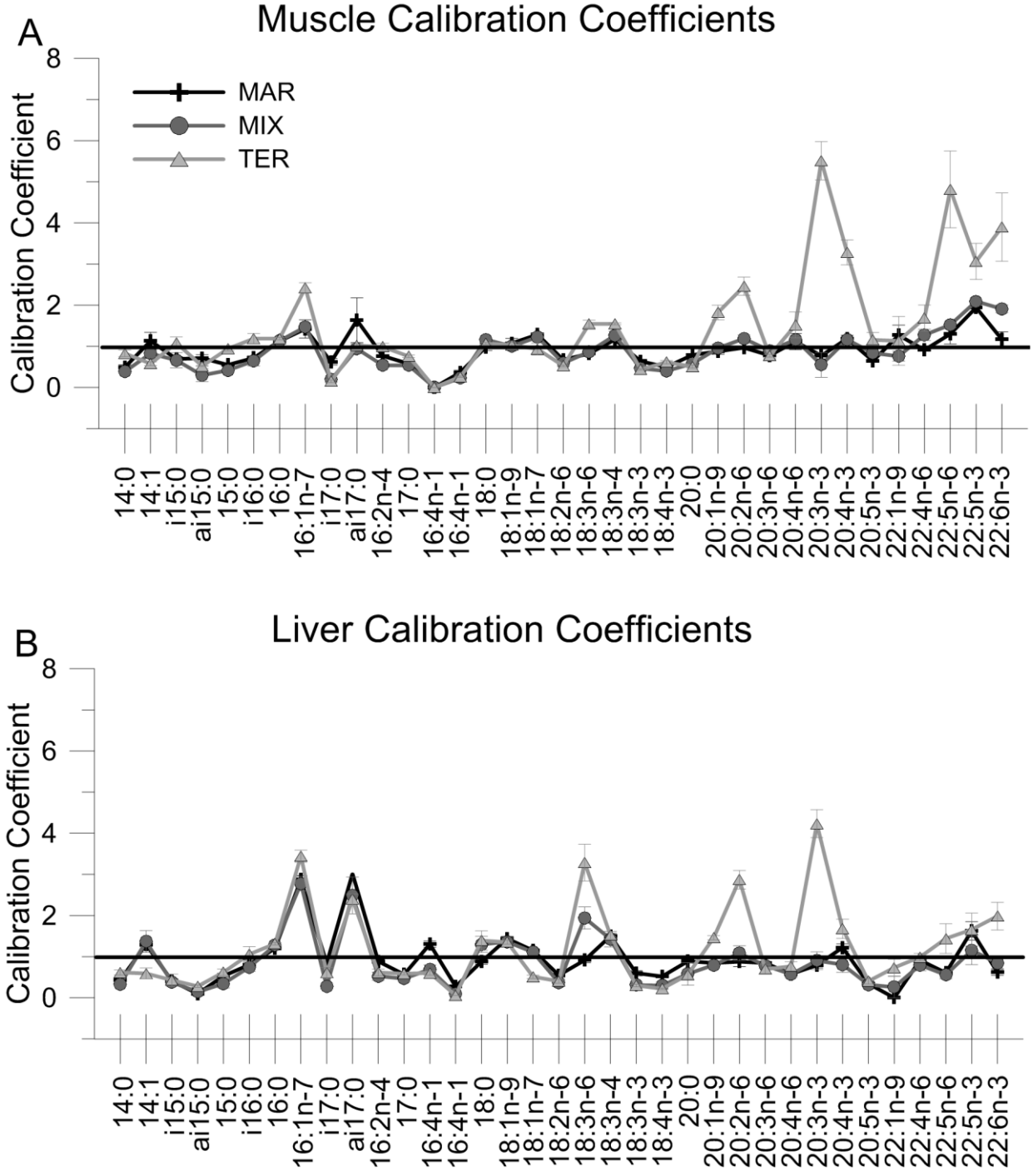

Figure 2.3. Fatty acid (FA) calibration coefficients ($\%FA_{tissue} \div \%FA_{diet}$; mean ± SE) for all identified FAs in muscle (A) and liver (B) of juvenile Atlantic croaker after feeding for 104 days on the marine (MAR, black cross), mixed (MIX, dark grey circle) or terrestrial (TER, light grey triangle) diets. n = 3 per diet treatment

Appendices

APPENDIX A: CHAPTER 1 SUPPLEMENTARY MATERIAL

As noted in Chapter 1, *Onisimus glacialis* samples for isotopic analysis were collected and prepared by Harris et al. (*in prep*) as follows: After collection, amphipods were rinsed and dried in aluminum dishes at 60°C, then immersed in 1 N HCl for several hours to remove carbonates. Specimens were then rinsed with distilled water and dried again. Only muscle tissue were analyzed for stable isotopes. Trophic levels (TLs) were calculated from nitrogen isotope values using the equation of trophic enrichment (Eq. A):

Equation A: $$TL = (\delta^{15}N_{consumer} - \delta^{15}N_{POM}) / 3.4 + 1$$

where, 3.4 is the average enrichment in $\delta^{15}N$ between TLs with POM as the primary carbon source in the Alaskan Arctic, as demonstrated by Iken et al. (2010).

Table A1. Carbon and nitrogen stable isotope values for *Onisimus glacialis* collected in April, June and August (see: Harris et al. *in prep*). Mean $\delta^{15}N$ values of particulate organic matter (POM) are site-specific and were collected from a parallel study by Connelly et al. (2015). Trophic level for *O. glacialis* was calculated using Eq. A. Final values are given as means and standard deviation (SD), except June (n=2) which is the half range.

Sample ID	Date Collected	Month	Year	Location	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Molar C/N	$\delta^{15}N$ of POM	Trophic Level
61	20-Apr-12	April	2012	AN	12.4	-21.4	11.7	6.8	2.5
63	20-Apr-12	April	2012	NU	10.2	-21.3	10.6	6.8	1.9
110	20-Apr-12	April	2012	NU	10.9	-20.1	11.4	6.8	2.1
108	20-Apr-12	April	2012	NU	10.7	-22.6	13.2	6.8	2.0
608, 609	22-Jun-13	June	2013	NU	6.5	-18.0	9.1	2.8	2.0
610, 611	22-Jun-13	June	2013	NU	11.7	-18.9	8.2	2.8	3.3
812, 813	17-Aug-11	August	2011	AN	11.6	-21.4	8.9	6.3	2.4
674, 675	11-Aug-13	August	2013	AN	13.5	-21.7	15.1	7.0	2.7
65	11-Aug-12	August	2012	JA	10.1	-16.6	10.2	6.3	2.0
88	15-Aug-12	August	2012	JA	7.7	-16.6	7.6	6.3	1.4
67	15-Aug-12	August	2012	JA	10.7	-21.4	12.7	6.3	2.1
658, 659	13-Aug-13	August	2013	JA	9.3	-18.7	7.7	7.0	1.6
842, 843	7-Aug-11	August	2011	JA	8.4	-22.5	14.5	6.3	1.5
142	10-Aug-13	August	2013	KA	11.1	-20.3	8.2	7.0	2.1
				April	11.0 ± 1.0	-21.4 ± 1.1	11.7 ± 1.1	-	2.1 ± 0.3
				June	9.1 ± 2.6	-18.5 ± 0.4	8.7 ± 0.5	-	2.7 ± 0.7
				August	10.3 ± 1.9	-19.9 ± 2.3	10.6 ± 3.1	-	2.0 ± 0.5

APPENDIX B: CHAPTER 2 SUPPLEMENTARY MATERIAL

Table B1. Fatty acid (FA) calibration coefficients ($\%FA_{tissue} \div \%FA_{diet}$; n = 9) for fatty acids >0.05% in muscle or liver tissues of Atlantic croaker fed three experimental diets for 104 days. Values are the means and SE across all three diet treatments.

Calibration Coefficients				
Fatty acid	Liver CC	SE	Muscle CC	SE
14:0	0.46	0.05	0.57	0.07
16:0	1.26	0.02	1.15	0.01
16:1n-7	3.03	0.13	1.77	0.18
*ai*17:0	2.64	0.21	1.20	0.19
16.2n-4	0.68	0.07	0.76	0.07
18:0	1.19	0.12	1.07	0.04
18:1n-9	1.38	0.04	1.04	0.04
18:1n-7	0.92	0.11	1.15	0.07
18:2n-6	0.44	0.04	0.59	0.02
18:3n-3	0.40	0.06	0.51	0.03
18:4n-3	0.35	0.05	0.50	0.04
20:1n-9	1.03	0.11	1.22	0.16
20:4n-6	0.64	0.05	1.27	0.13
20:4n-3	1.24	0.15	1.88	0.36
20:5n-3	0.35	0.03	0.88	0.09
22:1n-9	0.33	0.14	1.05	0.20
22:5n-3	1.48	0.18	2.37	0.22
22:6n-3	1.15	0.24	2.33	0.48

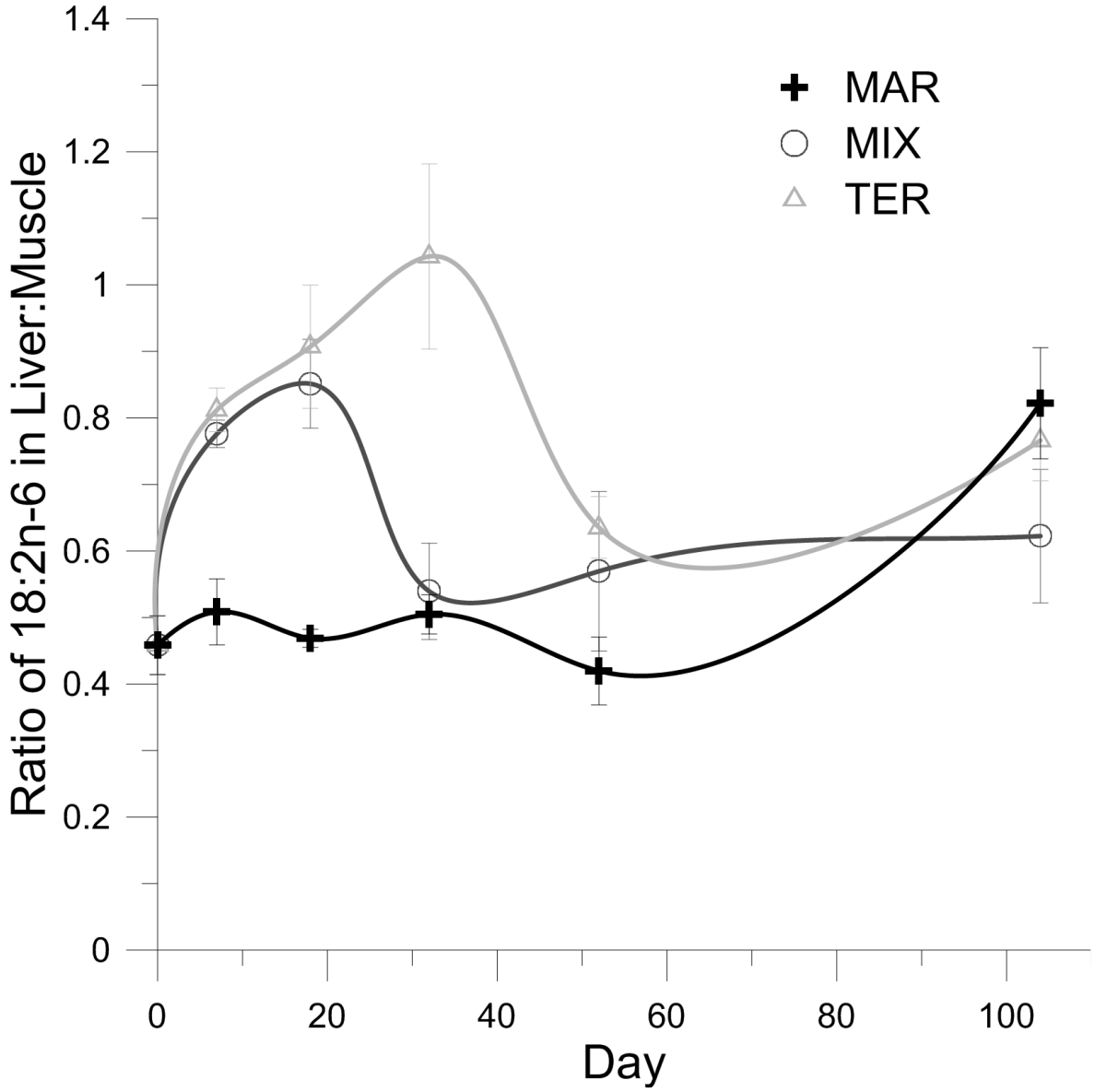

Figure B1. Ratio of 18:2n-6 (%) in liver to 18:2n-6 (%) in muscle of juvenile Atlantic croakers after feeding on a marine (MAR, cross), mixed (MIX, circles), or terrestrial (TER, grey triangles) for 104 days. Data are mean ± SE, fitted with a smooth spline. n = 3 per diet treatment.

APPENDIX C: FRACTIONATION FACTORS OF FATTY ACID-SPECIFIC ISOTOPES FOR TROPHIC STUDIES

C.1. Introduction

There are limitations to using bulk stable isotopes for ecological studies, which include isotopic routing and fractionation from diet to tissue (Bec et al., 2011). Bulk isotope measurements are ideal for broadly defining diet source (e.g. marine vs. terrestrial), but sources with similar signatures are not easily discernable (France et al., 1996). It is thought that fatty acid stable isotope analysis (FA-SIA) can fill this gap in knowledge, and enhance the ability of fatty acids and isotopes as source-specific tracers. But, in order for FA-SIA analysis to accurately characterize trophic sources, there are 2 main requirements. First, there must be distinct end members (i.e. FA isotope values must be distinctly different). For example, distinct end members have been demonstrated for certain FAs, like EPA (20:5n-3) in the Arctic, where distinct isotope values of EPA were used to distinguish between ice-algae and pelagic phytoplankton (e.g. Wang et al., 2014a). Second, the particular FA must be incorporated from diet to consumer in a predictable way (i.e. consistent/known fractionation factors). Currently, much is still unknown about fractionation factors of individual FAs, and most field studies have assumed that FA are assimilated from diet to consumer completely unmodified. We hypothesize that the FA isotopes are fractionated, in a predictable way. The FA-SI values of Atlantic croaker liver and muscle tissue were investigated as an extension of the feeding study documented in Chapter 2, where fish were fed a marine (MAR), mixed (MIX) or terrestrial (TER) diet for 104 days.

C.2. Methods

Experimental design, procedure, and sample processing are detailed in Chapter 2. Diet feeds for FA-SI were sampled on day 0 and day 32 (n=2 per treatment). Muscle and liver tissues were sampled from the control diet at day 0 (n=2), and at day 104 (n=2 per treatment). Compound-specific isotope analysis (CSIA) was performed using the same aliquot of previously derivatized fatty acid methyl esters (FAMEs). The FAMEs dissolved in hexane were measured with a GC-combustion isotope ratio mass spectrometer (GC-C-IRMS) with a BPX70 column at the UC Davis

Stable Isotope Facility (University of California, USA). FAMEs were corrected for the addition of the methyl group by measuring the $\delta^{13}C$ value of the BF3-methanol (-53.7‰) used in the derivatization process. The fractional contribution of the methyl group in a FAME depends on its chain length, where **x** is the fractional carbon contribution of the free FA to the ester. For example, 18:2n-6 has an **x** of 18/19. The corrected $\delta^{13}C$ value of each FA was calculated with the equation (Abrajano et al., 1994):

Equation C1: $$\delta^{13}C_{FA} = \frac{(\delta^{13}C_{FAME} - (1-x) \times \delta^{13}C_{CH3OH}}{x}$$

Stable isotope ratios for FA-SI are calculated using δ-notation (Eq. B2) relative to the international standard for carbon, Vienna PeeDeeBelemnite (VPDB) ($^{13}C/^{12}C = 0.0112372$).

Equation C2: $$\delta^{13}C\ (‰) = \left(\frac{^{13}C}{^{12}C}\text{Sample} \div \frac{^{13}C}{^{12}C}\text{VPDB} - 1\right) \times 1000$$

Trophic discrimination factors (TDF; $\Delta^{13}C$ FA tissue - $\Delta^{13}C$ FA diet) and fractionation factors (α; $\delta^{13}C$ FA tissue / $\delta^{13}C$ FA diet) were calculated for liver and muscle tissue of each fish after 104 days of feeding (Table C.1). One-way ANOVA was used to compare fractionation factors (α) between liver and muscle of fish from the three diet treatments after 104 days of feeding (where $p > 0.05$ is significant). Post hoc testing was carried out using Tukey's test with an adjusted p-value of 0.017 (0.05/3). TDFs and fractionation factors from this study and all currently published controlled feeding studies with FA-SIA of four essential FAs (18:2n-6, 18:3n-3, 20:5n-3, and 22:6n-3) were compiled (Table C.2).

C.3. Results and Discussion

FA profiles of experimental diets and Atlantic croaker tissues are detailed and discussed in Chapter 2. The first requirement for using FA-SIA for accurate assessments of diet is that there must be endmembers with distinct isotope signatures. Although our sample size was not large enough (n=2) for robust statistical analysis, the error bars (standard deviation) show certain FAs where at least one diet feed was different from the other two (Fig. C.1). In particular, the TER diet had FA-SI values that did not overlap with MAR or MIX values for: 20:1n-9, 20:4n-6, and 22:6n-3. The FAs 16:1n9/7 and 20:5n-3 were distinctly different across all three treatments.

The second requirement is that FAs are incorporated from diet to tissue in a predictable way (i.e. regardless of diet, fractionation factors and/or trophic discrimination factors are not significantly different). TDF are expressed in Figure C.2. FAs with fractionation factors that were not significantly different amongst the three treatments include (liver: 18:1n-9, 20:1n-9, 20:5n-3; muscle: 16:0) (Table C.2). According to our experiment, the only FAs that meet both requirements are 20:1n-9 and 20:5n-3. Therefore, they are reliable trophic markers for Atlantic croaker FA-SI, and possibly for other species too. Notably, these two FAs were only consistent in the liver, a neutral-lipid (NL) rich organ. Therefore, 20:1n-9 and 20:5n-3 may only be reliable trophic markers for NL-rich (TAG-rich) tissues.

The question remains if other controlled feeding studies agree with our results, and if there is a possibility for a "universal fractionation factor". We compiled data from all currently known feeding studies that measure FA-SIs for essential FAs (18:2n-6, 18:3n-3, 20:5n-3, 22:6n-3), particularly in NL-rich tissue (Table C.2). This includes four studies, including ours, with TDF and fractionation factors calculated at the final experimental time point.

For 20:5n-3, the mean fractionation factor (α) in our study was 0.95±0.03‰ SD which was lower but within the range of the mean α for all 4 studies (0.99±0.04‰). Fractionation factors for 18:2n-6, 18:3n-3, 20:5n-3, and 22:6n-3 from all studies ranged from 4.09‰ to -2.10‰. The fractionation factors given herein may represent a starting point for future field applications. In practice, when a controlled feeding study is unavailable for a specific study organism, and only field data is collected, the mean fractionation factors for individual FAs could be applied, specifically to NL-rich tissue and/or NL extracts. While the results we present here are preliminary, a rough estimate of a "universal fractionation factor" would be 0.98 ± 0.05 SD, the mean α of all 4 essential FA reported in the four controlled studies (Table C.2). Given this, a slight depletion in FA-SI values can be expected from diet to tissue. A field study by Gladyshev et al., (2012) documented a FA-SI depletion with each trophic step, in a four trophic level aquatic food chain in the Yenisei River. Therefore, fractionation factors may need to be applied for each trophic level to obtain the original source-specific isotope value.

C.4. Conclusions

We demonstrated through our feeding experiment and other published experiments that there are consistent fractionation factors between diet and consumer for a given FA, particularly in NL-rich tissues. While it is apparent that more controlled feeding studies are needed to move the field forward (Bec et al., 2011), we also stress the need to create a "lipid library" (i.e. "fatty acid library") that clearly characterizes the FA-SI signatures for all possible primary producers in a system. From there, established fractionation factors can be used to determine dietary source.

Table C.1. Fractionation factors (α; δ^{13}C FA tissue / diet δ^{13}C FA) for liver and muscle tissue of Atlantic croaker after 104 days of feeding.

	Liver α								**Muscle α**							
	MAR		MIX		TER		Average		MAR		MIX		TER		Average	
14:0[1,2]	[a]1.00	(0.02)	[a]0.99	(0.06)	1.17	(0.02)	1.05	(0.09)	[c]0.99	(0.01)	[c]0.95	(0.06)	1.09	(0.01)	1.01	(0.07)
16:0[1]	[a]0.98	(0.01)	[a,b]1.02	(0.03)	[b]1.06	(0.01)	1.02	(0.04)	[c]0.96	(0.02)	[c]0.95	(0.03)	[c]0.98	(0.01)	**0.96**	**(0.03)**
16:1n-9/7[1,2]	[a]1.02	(0.01)	[a]1.07	(0.03)	1.23	(0.04)	1.11	(0.10)	[c]1.04	(0.02)	[c]1.05	(0.04)	1.19	(0.03)	1.09	(0.08)
18:0[1,2]	[a]1.00	(0.02)	[a]1.02	(0.03)	1.15	(0.02)	1.06	(0.07)	[c]0.99	(0.02)	[c]0.99	(0.03)	1.07	(0.01)	1.02	(0.05)
18:1n-9[2]	[a]1.01	(0.01)	[a]0.98	(0.05)	[a]0.96	(0.10)	**0.98**	**(0.06)**	[c]1.03	(0.03)	[d]0.95	(0.02)	[c,d]1.00	(0.04)	0.99	(0.04)
18:2n-6[1,2]	[a]1.00	(0.02)	0.91	(0.03)	[a]0.98	(0.01)	0.96	(0.05)	[c]1.00	(0.01)	0.93	(0.03)	[c]0.98	(0.00)	0.97	(0.04)
18:3n-3[1,2]	[a]0.98	(0.03)	0.88	(0.03)	[a]1.00	(0.02)	0.96	(0.06)	[c]1.05	(0.03)	0.92	(0.03)	[c]0.99	(0.02)	0.99	(0.06)
20:1n-9[2]	[a]1.02	(0.01)	[a]0.95	(0.07)	[a]0.99	(0.03)	**0.99**	**(0.05)**	[c]1.00	(0.04)	[c]0.94	(0.07)	0.70	(0.02)	0.88	(0.14)
20:4n-6[1,2]	[a]1.00	(0.01)	[b]0.94	(0.01)	[a,b]0.99	(0.02)	0.97	(0.03)	[c]0.84	(0.16)	[c]0.96	(0.12)	[c]1.10	(0.03)	0.97	(0.16)
20:5n-3[2]	[a]0.94	(0.02)	[a]0.95	(0.04)	[a]0.97	NA	**0.95**	**(0.03)**	0.91	(0.01)	1.02	(0.02)	1.11	NA	0.98	(0.08)
22:6n-3[1,2]	[a]0.94	(0.01)	[a]0.96	(0.03)	1.03	(0.01)	0.98	(0.05)	[c]0.95	(0.01)	[c,d]0.98	(0.03)	[d]1.04	(0.02)	0.99	(0.05)

Values are mean (SD), where n = 4 per treatment, except TER 20:5n-3 with n = 1. Bold values denote a combined mean α from treatments that were not significantly different ($p > 0.05$).

[1]Liver α of all three treatments are significantly different ($p < 0.05$), according to results of one-way ANOVA.

[2]Muscle α values all three treatments significantly different ($p < 0.05$), according to results of one-way ANOVA.

The same letters (a-d) indicate statistically similar values among tissue treatments. All other comparisons are significantly different according to Tukey's test with adjusted p value (0.017).

Table C.2. Summary of mean trophic discrimination factors ($\delta^{13}C$) and fractionation factors (α) of fatty acid carbon isotopes in controlled diet studies. All data comes from neutral lipid-rich tissue or neutral lipid extractions. NA denotes when sample size was not available.

Table C.2.

Study	FA	Species	Tissue	Diet Type	Δ13C tissue - diet	α tissue / diet	n
Budge et al 2011	18:2n-6	Steller's eider	Adipose	diet 1	1.90	0.93	NA
Budge et al 2011	18:2n-6	Spectacled eider	Adipose	diet 1	2.13	0.93	NA
Our study	18:2n-6	Atlantic croaker	Liver	MAR	-0.09	1.00	4
Our study	18:2n-6	Atlantic croaker	Liver	MIX	2.96	0.91	4
Our study	18:2n-6	Atlantic croaker	Liver	TER	0.60	0.98	4
Hixon et al 2014	18:2n-6	Rainbow trout	Muscle	Fish oil	3.40	0.89	9
Hixon et al 2014	18:2n-6	Rainbow trout	Muscle	Camelina oil	-2.10	1.07	9
Budge et al 2011	18:3n-3	Steller's eider	Adipose	diet 1	1.69	0.95	NA
Budge et al 2011	18:3n-3	Spectacled eider	Adipose	diet 1	1.32	0.96	NA
Our study	18:3n-3	Atlantic croaker	Liver	MAR	0.53	0.98	4
Our study	18:3n-3	Atlantic croaker	Liver	MIX	4.09	0.88	4
Our study	18:3n-3	Atlantic croaker	Liver	TER	-0.15	1.00	4
Hixon et al 2014	18:3n-3	Rainbow trout	Muscle	Fish oil	2.70	0.92	9
Hixon et al 2014	18:3n-3	Rainbow trout	Muscle	Camelina oil	-1.80	1.05	9
Bec et al 2011	20:5n-3	Daphnia	Whole animal NL	diatom	-1.30	1.04	NA
Bec et al 2011	20:5n-3	Daphnia	Whole animal NL	flagellate	-0.10	1.00	NA
Budge et al 2011	20:5n-3	Steller's eider	Adipose	diet 1	-0.53	1.02	NA
Budge et al 2011	20:5n-3	Spectacled eider	Adipose	diet 1	-0.52	1.02	NA
Our study	20:5n-3	Atlantic croaker	Liver	MAR	1.73	0.94	4
Our study	20:5n-3	Atlantic croaker	Liver	MIX	1.31	0.95	4
Our study	20:5n-3	Atlantic croaker	Liver	TER	0.87	0.97	4
Hixon et al 2014	20:5n-3	Rainbow trout	Muscle	Fish oil	-0.30	1.01	9
Budge et al 2011	22:6n-3	Steller's eider	Adipose	diet 1	0.84	0.97	NA
Budge et al 2011	22:6n-3	Spectacled eider	Adipose	diet 1	0.77	0.97	NA
Our study	22:6n-3	Atlantic croaker	Liver	MAR	1.65	0.94	4
Our study	22:6n-3	Atlantic croaker	Liver	MIX	0.98	0.96	4
Our study	22:6n-3	Atlantic croaker	Liver	TER	-0.84	1.03	4
Hixon et al 2014	22:6n-3	Rainbow trout	Muscle	Fish oil	-1.10	1.04	9

Table C.2. Continued.

		Average Δ13C	**SD**	**SE**	**Average α**	**SD**	**SE**
	18:2n-6	1.26	1.92	0.73	0.96	0.06	0.02
	18:3n-3	1.20	1.92	0.73	0.96	0.06	0.02
	20:5n-3	0.15	1.05	0.37	0.99	0.04	0.01
	22:6n-3	0.38	1.10	0.45	0.99	0.04	0.02
	All 4 PUFA	**0.74**	1.55	0.29	**0.98**	0.05	0.01

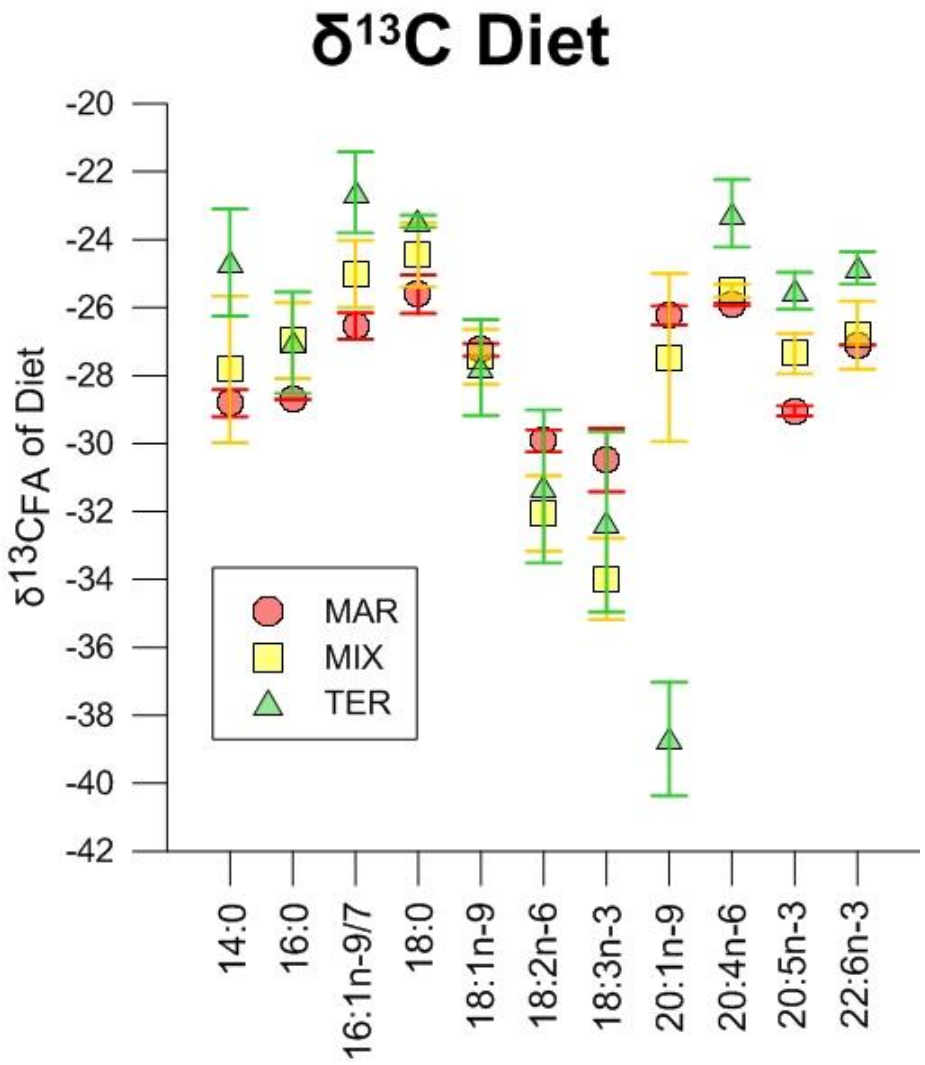

Figure C.1. Carbon isotope values of individual fatty acids in three diets. Data are mean and standard deviation.

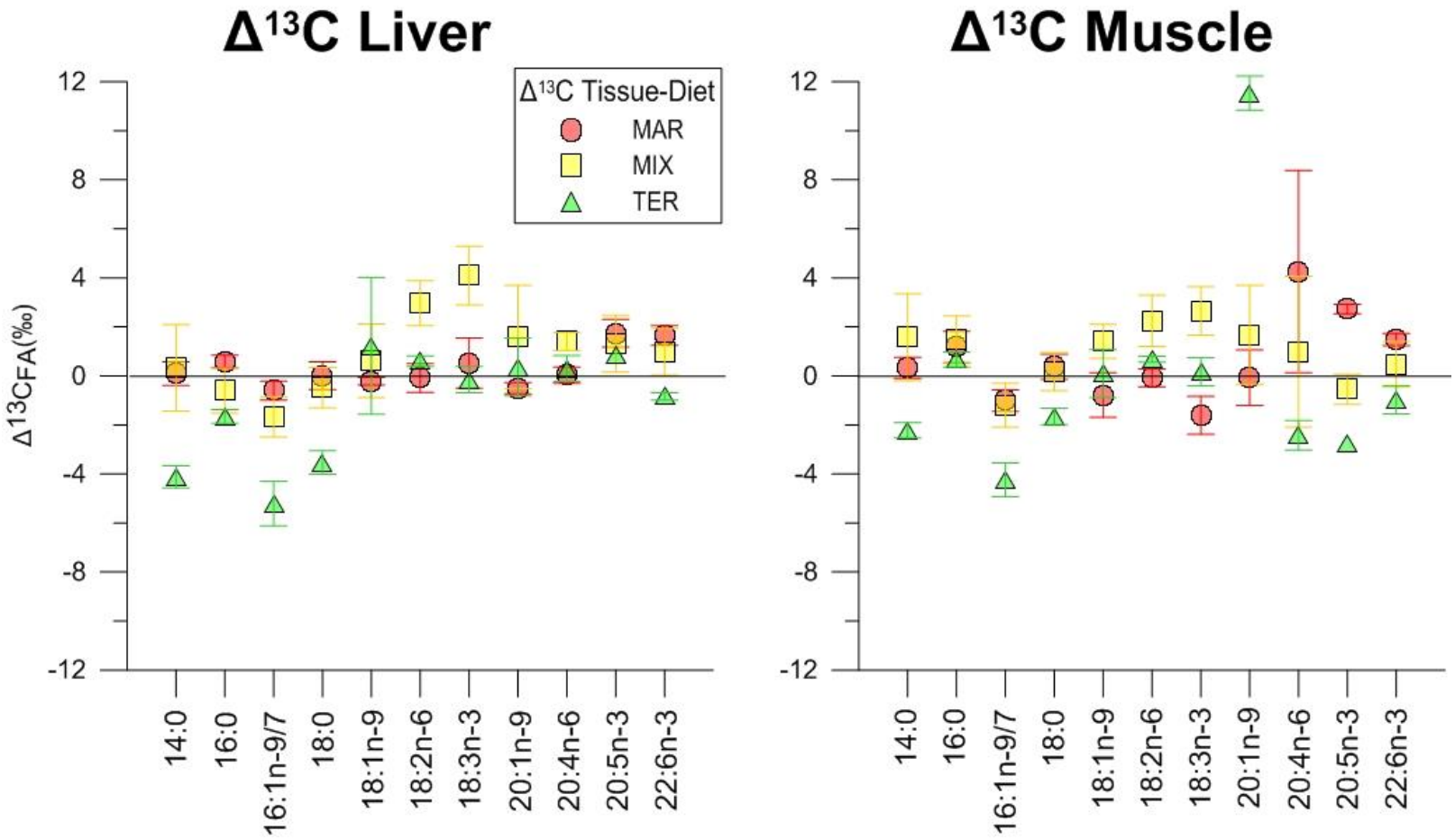

Figure C.2. Trophic discrimination factors (TDF) of carbon isotope values of individual fatty acids in Atlantic croaker tissue after 104 of feeding.

References

Abrajano, T.A., Murphy, D.E., Fang, J., Comet, P., Brooks, J.M., 1994. 13C/12C ratios in individual fatty acids of marine mytilids with and without bacterial symbionts. Organic Geochemistry 21, 611–617. doi:10.1016/0146-6380(94)90007-8

Allan, E.L., Ambrose, S.T., Richoux, N.B., Froneman, P.W., 2010. Determining spatial changes in the diet of nearshore suspension-feeders along the South African coastline: Stable isotope and fatty acid signatures. Estuarine, Coastal and Shelf Science 87, 463–471. doi:10.1016/j.ecss.2010.02.004

Ando, S., Mori, Y., Nakamura, K., Sugawara, A., 1993. Characteristics of Lipid Accumulation Types in Five Species of Fish. Nippon Suisan Gakkaishi 59, 1559–1564. doi:10.2331/suisan.59.1559

Arndt, C.E., Beuchel, F., 2006. Life history and population dynamics of the Arctic sympagic amphipods *Onisimus nanseni* Sars and *O. glacialis* Sars (Gammaridea: Lysianassidae). Polar Biology 29, 239–248. doi:10.1007/s00300-005-0045-x

Ayaz, F.A., Olgun, A., 2000. Fatty acid composition in leaf lipids of some *Carex* L. (Cyperaceae) species from Northeast Anatolia (Turkey)JF - Grasas y Aceites; Vol 51, No 5 (2000).

Bec, A., Perga, M.-E., Koussoroplis, A., Bardoux, G., Desvilettes, C., Bourdier, G., Mariotti, A., 2011. Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies. Methods in Ecology and Evolution 2, 651–659. doi:10.1111/j.2041-210X.2011.00111.x

Bell, J.G., Wolfgang, K., 2010. Lipids in Aquafeeds, in: Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds. CRC Press, pp. 21–59. doi:doi:10.1201/9781439808634-c2 10.1201/9781439808634-c2

Benoit, D., Simard, Y., Gagné, J., Geoffroy, M., Fortier, L., 2010. From polar night to midnight sun: photoperiod, seal predation, and the diel vertical migrations of polar cod (*Boreogadus saida*) under landfast ice in the Arctic Ocean. Polar Biology 33, 1505–1520. doi:10.1007/s00300-010-0840-x

Böhm, M., Schultz, S., Koussoroplis, A.-M., Kainz, M.J., 2014. Tissue-specific fatty acids response to different diets in common carp (*Cyprinus carpio* L.). PloS one 9, e94759. doi:10.1371/journal.pone.0094759

Boschker, H.T.S., Kromkamp, J.C., Middelburg, J.J., 2005. Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in a

turbid, tidal estuary. Limnology and Oceanography 50, 70–80. doi:10.4319/lo.2005.50.1.0070

Bowen, W.D., Iverson, S.J., 2012. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29, 719–754. doi:10.1111/j.1748-7692.2012.00604.x

Bradstreet, M., 1986. Aspects of the biology of Arctic Cod (*Boreogadus saida*) and its importance in arctic marine food chains. Ministère des approvisionnements et Services., Ottawa.

Bradstreet, M.S.W., Cross, W.E., 1982. Trophic Relationships at High Arctic Ice Edges. Arctic 35, 1–12. doi:10.2307/40509298

Brown, S.C., 2006. Arctic wings: birds of the Arctic National Wildlife Refuge. Birds of the Arctic National Wildlife Refuge.

Budge, S., Parrish, C., Mckenzie, C., 2001. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. Marine Chemistry 76, 285–303. doi:10.1016/S0304-4203(01)00068-8

Budge, S.M., Iverson, S.J., Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. Marine Mammal Science 22, 759–801. doi:10.1111/j.1748-7692.2006.00079.x

Budge, S.M., Parrish, C.C., 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Organic Geochemistry 29, 1547–1559. doi:http://dx.doi.org/10.1016/S0146-6380(98)00177-6

Budge, S.M., Penney, S.N., Lall, S.P., 2011. Response of tissue lipids to diet variation in Atlantic salmon (*Salmo salar*): Implications for estimating diets with fatty acid analysis. Journal of Experimental Marine Biology and Ecology 409, 267–274. doi:10.1016/j.jembe.2011.09.002

Budge, S.M., Penney, S.N., Lall, S.P., Trudel, M., 2012. Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. Canadian Journal of Fisheries and Aquatic Sciences 69, 1033–1046. doi:10.1139/f2012-039

Budge, S.M., Wooller, M.J., Springer, A.M., Iverson, S.J., McRoy, C.P., Divoky, G.J., 2008. Tracing carbon flow in an arctic marine food web using fatty acid-stable isotope analysis. Oecologia 157, 117–29. doi:10.1007/s00442-008-1053-7

Carey, A.G., Boudrias, M.A., 1987. Feeding ecology of *Pseudalibrotus* (=*Onisimus*) *litoralis* Kroyer (Crustacea: Amphipoda) on the Beaufort Sea inner continental shelf. Polar Biology 8, 29–33. doi:10.1007/BF00297161

Carmack, E., Barber, D., Christensen, J., Macdonald, R., Rudels, B., Sakshaug, E., 2006. Climate variability and physical forcing of the food webs and the carbon budget on panarctic shelves. Progress in Oceanography 71, 145–181. doi:10.1016/j.pocean.2006.10.005

Carreón-Palau, L., Parrish, C.C., del Angel-Rodríguez, J.A., Pérez-España, H., Aguiñiga-García, S., 2013. Revealing organic carbon sources fueling a coral reef food web in the Gulf of Mexico using stable isotopes and fatty acids. Limnology and Oceanography 58, 593–612. doi:10.4319/lo.2013.58.2.0593

Castledine, A., Buckley, J., 1980. Distribution and mobility of omega 3 fatty acids in rainbow trout fed varying levels and types of dietary lipid. The Journal of nutrition.

Claustre, H., Marty, J.C., Cassiani, L., Dagaut, J., 1988. Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea (France): Ecological implications. Marine Microbial Food Webs 3, 51–66.

Cober, E.R., Frégeau-Reid, J.A., Butler, G., Voldeng, H.D., 2006. Genotype–Environment Analysis of Parameters Describing Water Uptake in Natto Soybean. Crop Science 46, 2415. doi:10.2135/cropsci2006.02.0069

Connelly, T., McClelland, J., Crump, B., Kellogg, C., Dunton, K., 2015. Seasonal changes in quantity and composition of suspended particulate organic matter in lagoons of the Alaskan Beaufort Sea. Marine Ecology Progress Series 527, 31–45. doi:10.3354/meps11207

Connelly, T.L., Deibel, D., Parrish, C.C., 2014. Trophic interactions in the benthic boundary layer of the Beaufort Sea shelf, Arctic Ocean: Combining bulk stable isotope and fatty acid signatures. Progress in Oceanography 120, 79–92. doi:http://dx.doi.org/10.1016/j.pocean.2013.07.032

Copeman, L., Parrish, C., Gregory, R., Jamieson, R., Wells, J., Whiticar, M., 2009. Fatty acid biomarkers in coldwater eelgrass meadows: elevated terrestrial input to the food web of age-0 Atlantic cod *Gadus morhua*. Marine Ecology Progress Series 386, 237–251. doi:10.3354/meps08063

Copeman, L.A., Laurel, B.J., Parrish, C.C., 2013. Effect of temperature and tissue type on fatty acid signatures of two species of North Pacific juvenile gadids: A laboratory

feeding study. Journal of Experimental Marine Biology and Ecology 448, 188–196. doi:10.1016/j.jembe.2013.07.008

Copeman, L.A., Parrish, C.C., 2004. Lipid classes, fatty acids, and sterols in seafood from Gilbert Bay, southern labrador. Journal of agricultural and food chemistry 52, 4872–81. doi:10.1021/jf034820h

Craig, P.C., Griffiths, W.B., Haldorson, L., McElderry, H., 1982. Ecological Studies of Arctic Cod (*Boreogadus saida*) in Beaufort Sea Coastal Waters, Alaska. Canadian Journal of Fisheries and Aquatic Sciences 39, 395–406. doi:10.1139/f82-057

Cushing, D.H., 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. Journal of Plankton Research 11, 1–13. doi:10.1093/plankt/11.1.1

Dalsgaard, J., St. John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46, 225–340. doi:10.1016/S0065-2881(03)46005-7

Dittmar, T., Kattner, G., 2003. The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. Marine Chemistry 83, 103–120. doi:10.1016/S0304-4203(03)00105-1

Dunstan, G.A., Volkman, J.K., Jeffrey, S.W., Barrett, S.M., 1992. Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. Journal of Experimental Marine Biology and Ecology 161, 115–134. doi:10.1016/0022-0981(92)90193-E

Dunton, K., Saupe, S., Golikov, A., Schell, D., Schonberg, S., 1989. Trophic relationships and isotopic gradients among arctic and subarctic marine fauna. Marine Ecology Progress Series. doi:10.3354/meps056089

Dunton, K., Schonberg, S., Cooper, L., 2012. Food Web Structure of the Alaskan Nearshore Shelf and Estuarine Lagoons of the Beaufort Sea. Estuaries and Coasts 35, 416–435. doi:10.1007/s12237-012-9475-1

Dunton, K.H., Schell, D.M., 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an arctic kelp community: δ13C evidence. Marine Biology 93, 615–625. doi:10.1007/BF00392799

Dunton, K.H., Weingartner, T., Carmack, E.C., 2006. The nearshore western Beaufort Sea ecosystem: Circulation and importance of terrestrial carbon in arctic coastal

food webs. Progress in Oceanography 71, 362–378. doi:10.1016/j.pocean.2006.09.011

Egeler, O., Williams, T.D., 2000. Seasonal, age, and sex-realted variation in fatty-acid composition of depot fat in relation to migration in western sandpipers. The Auk 117, 110. doi:10.1642/0004-8038(2000)117[0110:SAASRV]2.0.CO;2

Ejdung, G., Elmgren, R., 2001. Predation by the benthic isopod Saduria entomon on two Baltic Sea deposit-feeders, the amphipod *Monoporeia affinis* and the bivalve *Macoma balthica*. Journal of Experimental Marine Biology and Ecology 266, 165–179. doi:10.1016/S0022-0981(01)00353-7

Falk-Petersen, S., Mayzaud, P., Kattner, G., Sargent, J.R., 2009. Lipids and life strategy of Arctic Calanus. Marine Biology Research 5, 18–39. doi:10.1080/17451000802512267

Falk-Petersen, S., Sargent, J.R., Tande, K.S., 1987. Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biology 8, 115–120. doi:10.1007/BF00297065

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

France, R.L., 1996. Scope for use of stable carbon isotopes in discerning the incorporation of forest detritus into aquatic foodwebs. Hydrobiologia 325, 219–222. doi:10.1007/BF00014987

Fry, B., Arnold, C., 1982. Rapid 13C/12C turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia 54, 200–204. doi:10.1007/BF00378393

Gallagher, M.L., Harrell, M.L., Rulifson, R.A., 1991. Variation in lipid and fatty-acid contents of Atlantic croakers, Stiped mullet, and Summer flound. Transactions of the American Fisheries Society (1900) 120, 614–619.

Galloway, A.W.E., Britton-Simmons, K.H., Duggins, D.O., Gabrielson, P.W., Brett, M.T., 2012. Fatty acid signatures differentiate marine macrophytes at ordinal and family ranks. Journal of Phycology 48, 956–965. doi:10.1111/j.1529-8817.2012.01173.x

Gladyshev, M.I., Sushchik, N.N., Kalachova, G.S., Makhutova, O.N., 2012. Stable isotope composition of fatty acids in organisms of different trophic levels in the Yenisei River. PloS one 7, e34059. doi:10.1371/journal.pone.0034059

Goñi, M. a., Yunker, M.B., Macdonald, R.W., Eglinton, T.I., 2005. The supply and preservation of ancient and modern components of organic carbon in the Canadian Beaufort Shelf of the Arctic Ocean. Marine Chemistry 93, 53–73. doi:10.1016/j.marchem.2004.08.001

Gradinger, R., Bluhm, B., 2010. Timing of ice algal grazing by the Arctic nearshore benthic amphipod *Onisimus litoralis*. Arctic 63, 355–358.

Graeve, M., Kattner, G., Wiencke, C., Ulf Karsten, 2002. Fatty acid composition of Arctic and Antarctic macroalgae: indicator of phylogenetic and trophic relationships. Marine Ecology Progress Series 231, 67–74.

Graeve, M., Albers, C., Kattner, G., 2005. Assimilation and biosynthesis of lipids in Arctic Calanus species based on feeding experiments with a 13C labelled diatom. Journal of Experimental Marine Biology and Ecology 317, 109–125. doi:10.1016/j.jembe.2004.11.016

Graeve, M., Kattner, G., Piepenburg, D., 1997. Lipids in Arctic benthos: does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biology 18, 53–61. doi:10.1007/s003000050158

Graham, C., Oxtoby, L., Wang, S.W., Budge, S.M., Wooller, M.J., 2014. Sourcing fatty acids to juvenile polar cod (*Boreogadus saida*) in the Beaufort Sea using compound-specific stable carbon isotope analyses. Polar Biology 37, 697–705. doi:10.1007/s00300-014-1470-5

Guil-Guerrero, J.L., Venegas-Venegas, E., Rincón-Cervera, M.Á., Suárez, M.D., 2011. Fatty acid profiles of livers from selected marine fish species. Journal of Food Composition and Analysis 24, 217–222. doi:http://dx.doi.org/10.1016/j.jfca.2010.07.011

Harris, C., McClelland, J., Crump, B., Connelly, T., Dunton, K., *In Prep*. Terrestrial subsidies and trophic structure of eastern Alaskan Beaufort Lagoons.

Hesslein, R.H., Hallard, K.A., Ramlal, P., 1993. Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (*Coregonus nasus*) in Response to a Change in Diet Traced by δ34S, δ13C, and δ15N. Canadian Journal of Fisheries and Aquatic Sciences 50, 2071–2076. doi:10.1139/f93-230

Hietala, T., Hiekkala, P., Rosenqvist, H., Laakso, S., Tahvanainen, L., Repo, T., 1998. Fatty acid and alkane changes in willow during frost-hardening. Phytochemistry 47, 1501–1507. doi:10.1016/S0031-9422(97)01083-2

Hobson, K. a., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., Fortier, M., 2002. A stable isotope (d13C, d15N) model for the North Water food web: Implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Research Part II: Topical Studies in Oceanography 49, 5131–5150. doi:10.1016/S0967-0645(02)00182-0

Holmes, R.M., McClelland, J.W., Raymond, P.A., Frazer, B.B., Peterson, B.J., Stieglitz, M., 2008. Lability of DOC transported by Alaskan rivers to the Arctic Ocean. Geophysical Research Letters 35, L03402. doi:10.1029/2007GL032837

Horner, R., Schrader, G.C., 1982. Relative Contributions of Ice Algae, Phytoplankton, and Benthic Microalgae to Primary Production in Nearshore Regions of the Beaufort Sea. ARCTIC.

Iverson, S., Field, C., Bowen, W.D., Blanchard, W., 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs 74, 211–235.

Iverson, S.J., 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination, in: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer New York, pp. 281–308.

Jeffrey, S.W., 1976. A report of green algal pigments in the Central North Pacific Ocean. Marine Biology 37, 33–37. doi:10.1007/BF00386776

Kaufman, M.R., Gradinger, R.R., Bluhm, B. a., O'Brien, D.M., 2008. Using stable isotopes to assess carbon and nitrogen turnover in the Arctic sympagic amphipod *Onisimus litoralis*. Oecologia 158, 11–22. doi:10.1007/s00442-008-1122-y

Kelly, J., Scheibling, R., 2012. Fatty acids as dietary tracers in benthic food webs. Marine Ecology Progress Series 446, 1–22. doi:10.3354/meps09559

Koussoroplis, A., Bec, a, Perga, M., Koutrakis, E., Desvilettes, C., Bourdier, G., 2010. Nutritional importance of minor dietary sources for leaping grey mullet *Liza saliens* (Mugilidae) during settlement: insights from fatty acid δ13C analysis. Marine Ecology Progress Series 404, 207–217. doi:10.3354/meps08485

Kukliski, P., Porter, J.S., 2004. *Alcyonidium disciforme*: an exceptional Arctic bryozoan. Journal of the Marine Biological Association of the UK 84, 267–275. doi:10.1017/S0025315404009130h

Kumari, P., Bijo, A.J., Mantri, V.A., Reddy, C.R.K., Jha, B., 2013. Fatty acid profiling of tropical marine macroalgae: an analysis from chemotaxonomic and nutritional perspectives. Phytochemistry 86, 44–56. doi:10.1016/j.phytochem.2012.10.015

Legezyńska, J., Kedra, M., Walkusz, W., 2014. Identifying trophic relationships within the high Arctic benthic community: How much can fatty acids tell? Marine Biology 161, 821–836. doi:10.1007/s00227-013-2380-8

Leonardsson, K., 1991. Effects of cannibalism and alternative prey on population dynamics of *Saduria entomon* (Isopoda). Ecology 72, 1273–1285.

Léveillé, J., Amblard, C., Bourdier, G., 1997. Fatty acids as specific algal markers in a natural lacustrian phytoplankton. Journal of Plankton. 19, 469–490.

Litz, M., Brodeur, R., Emmett, R., Heppell, S., Rasmussen, R., O'Higgins, L., Morris, M., 2010. Effects of variable oceanographic conditions on forage fish lipid content and fatty acid composition in the northern California Current. Marine Ecology Progress Series 405, 71–85. doi:10.3354/meps08479

Litzow, M., Bailey, K., Prahl, F., Heintz, R., 2006. Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. Marine Ecology Progress Series 315, 1–11. doi:10.3354/meps315001

Lowry, L.F., Burns, J.J., 1980. Foods utilized by bowhead whales near Barter Island, Alaska, autumn 1979. Marine Fisheries Review 42, 88–91.

Magnone, L., Bessonart, M., Rocamora, M., Gadea, J., Salhi, M., 2015. Diet estimation of *Paralichthys orbignyanus* in a coastal lagoon via quantitative fatty acid signature analysis. Journal of Experimental Marine Biology and Ecology 462, 36–49. doi:10.1016/j.jembe.2014.10.008

Mayzaud, P., Boutoute, M., Noyon, M., Narcy, F., Gasparini, S., 2013. Lipid and fatty acids in naturally occurring particulate matter during spring and summer in a high arctic fjord (Kongsfjorden, Svalbard). Marine Biology 160, 383–398. doi:10.1007/s00227-012-2095-2

Mayzaud, P., Boutoute, M., 2015. Dynamics of lipid and fatty acid composition of the hyperiid amphipod *Themisto*: a bipolar comparison with special emphasis on seasonality. Polar Biology. doi:10.1007/s00300-015-1666-3

McClelland, J.W., Townsend-Small, A., Holmes, R.M., Pan, F., Stieglitz, M., Khosh, M., Peterson, B.J., 2014. River export of nutrients and organic matter from the North

Slope of Alaska to the Beaufort Sea. Water Resources Research 50, 1823–1839. doi:10.1002/2013WR014722

McKinney, M.A., Iverson, S.J., Fisk, A.T., Sonne, C., Rigét, F.F., Letcher, R.J., Arts, M.T., Born, E.W., Rosing-Asvid, A., Dietz, R., 2013. Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. Global change biology 19, 2360–72. doi:10.1111/gcb.12241

McLeod, R., Hyndes, G., Hurd, C., Frew, R., 2013. Unexpected shifts in fatty acid composition in response to diet in a common littoral amphipod. Marine Ecology Progress Series 479, 1–12. doi:10.3354/meps10327

Møller, P., 2006. Lipids and stable isotopes in marine food webs in West Greenland: Trophic relations and health implications. dmudk.

Mohan, J.A., S.D. Smith, T.L. Connelly, J. McClelland, S.Z. Herzka, B.D. Walther (*In Review*) Tissue-specific isotopic turnover and discrimination factors are affected by diet quality and lipid content in a marine consumer.

Morata, N., Renaud, P., Brugel, S., Hobson, K., Johnson, B., 2008. Spatial and seasonal variations in the pelagic–benthic coupling of the southeastern Beaufort Sea revealed by sedimentary biomarkers. Marine Ecology Progress Series 371, 47–63. doi:10.3354/meps07677

Müller-Navarra, D.C., Brett, M.T., Liston, A.M., Goldman, C.R., 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. Nature 403, 74–7. doi:10.1038/47469

Müller-Navarra, D.C., Brett, M.T., Park, S., Chandra, S., Ballantyne, A.P., Zorita, E., Goldman, C.R., 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427, 69–72. doi:10.1038/nature02210

Nanton, D.A., Vegusdal, A., Rørå, A.M.B., Ruyter, B., Baeverfjord, G., Torstensen, B.E., 2007. Muscle lipid storage pattern, composition, and adipocyte distribution in different parts of Atlantic salmon (*Salmo salar*) fed fish oil and vegetable oil. Aquaculture 265, 230–243. doi:10.1016/j.aquaculture.2006.03.053

Napolitano, G.E., 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems, in: Arts, M.T., Wainman, B.C. (Eds.), Lipids in Freshwater Ecosystems. New York, p 21–44.

Nicolaus, M., Petrich, C., Hudson, S.R., Granskog, M.A., 2013. Variability of light transmission through Arctic land-fast sea ice during spring. The Cryosphere 7, 977–986. doi:10.5194/tc-7-977-2013

Nye, J.A., Loewensteiner, D.A., Miller, T.J., 2010. Annual, Seasonal, and Regional Variability in Diet of Atlantic Croaker (*Micropogonias undulatus*) in Chesapeake Bay. Estuaries and Coasts 34, 691–700. doi:10.1007/s12237-010-9348-4

Parrish, C.C., 1999. Determination of total lipid, lipid classes and fatty acids in aquatic samples. In: Arts, M.T., Wainman, B.C. (Eds.), Lipids in freshwater ecosystems. New York, NY, p 4−12

Parrish Abrajano, T. A., Budge, S. M., Helleur, R. J., Hudson, E. D., Pulchan, K., Ramos, C., C.C., 2000. Lipid and Phenolic Biomarkers in Marine Ecosystems: Analysis and Applications. The Handbook of Environmental Chemistry 5, 223.

Parrish, C., 2009. Essential fatty acids in aquatic food webs, in: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer New York, pp. 309–326. doi:10.1007/978-0-387-89366-2_13

Parrish, C.C., Whiticar, M., Puvanendran, V., 2007. Is w6 docosapentaenoic acid an essential fatty acid during early ontogeny in marine fauna? Limnology and Oceanography 52, 476–479. doi:10.4319/lo.2007.52.1.0476

Petrik, R., Levin, P.S., Stunz, G.W., Malone, J., 1999. Recruitment of Atlantic croaker, *Micropogonias undulatus*: Do postsettlement processes disrupt or reinforce initial patterns of settlement? Fishery Bulletin 97, 954–961.

Pimm, S., Lawton, J., Cohen, J., 1991. Food web patterns and their consequences. Nature.

Pomerleau, C., Ferguson, S.H., Walkusz, W., 2010. Stomach contents of bowhead whales (*Balaena mysticetus*) from four locations in the Canadian Arctic. Polar Biology 34, 615–620. doi:10.1007/s00300-010-0914-9

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152, 179–89. doi:10.1007/s00442-006-0630-x

Recks, M.A., Seaborn, G.T., 2008. Variation in fatty acid composition among nine forage species from a southeastern US estuarine and nearshore coastal ecosystem. Fish physiology and biochemistry 34, 275–87. doi:10.1007/s10695-007-9186-x

Rooker, J.R., Holt, S.A., Soto, M.A., Holt, G.J., 1998. Postsettlement Patterns of Habitat Use by Sciaenid Fishes in Subtropical Seagrass Meadows. Estuaries 21, 318. doi:10.2307/1352478

Rosen, D., Tollit, D., 2012. Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. Marine Ecology Progress Series 467, 263–276. doi:10.3354/meps09934

Sahu, A., Pancha, I., Jain, D., Paliwal, C., Ghosh, T., Patidar, S., Bhattacharya, S., Mishra, S., 2013. Fatty acids as biomarkers of microalgae. Phytochemistry 89, 53–8. doi:10.1016/j.phytochem.2013.02.001

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199. doi:10.1016/S0044-8486(99)00083-6

Schell, D., 1983. Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: delayed production from peat in arctic food webs. Science 219, 1068–1071.

Schlechtriem, C., Arts, M.T., Johannsson, O.E., 2008. Effect of Long-term Fasting on the Use of Fatty Acids as Trophic Markers in the Opossum Shrimp *Mysis relicta*—A Laboratory Study. Journal of Great Lakes Research 34, 143–152. doi:10.3394/0380-1330(2008)34[143:EOLFOT]2.0.CO;2

Schultz, D.M., Quinn, J.G., 1973. Fatty acid composition of organic detritus from *Spartina alterniflora*. Estuarine and Coastal Marine Science 1, 177–190. doi:10.1016/0302-3524(73)90068-6

Spetzman, L.A., 1959. Vegetation of the Arctic Slope of Alaska, USGS Professional Paper 302-B. Washington.

Smith, S., 2012. Fractionation of Dissolved Solutes and Chromophoric Dissolved Organic Matter During Experimental Sea Ice Formation. Honors and Undergraduate Research. Available electronically from http : / /hdl .handle .net /1969 .1/148815.

St. John, M., Lund, T., 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Marine Ecology-Progress Series.

Stevens, C.J., Deibel, D., Parrish, C.C., 2004. Species-specific differences in lipid composition and omnivory indices in Arctic copepods collected in deep water

during autumn (North Water Polynya). Marine Biology 144, 905–915. doi:10.1007/s00227-003-1259-5

Stübing, D., Hagen, W., 2003. Fatty acid biomarker ratios - Suitable trophic indicators in Antarctic euphausiids? Polar Biology 26, 774–782.

Suzuki, K., Minami, C., Liu, H., Saino, T., 2002. Temporal and spatial patterns of chemotaxonomic algal pigments in the subarctic Pacific and the Bering Sea during the early summer of 1999. Deep Sea Research Part II: Topical Studies in Oceanography 49, 5685–5704. doi:10.1016/S0967-0645(02)00218-7

Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research 41, 717–732. doi:10.1111/j.1365-2109.2008.02150.x

Tocher, D.R., 2003. Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. Reviews in Fisheries Science 11, 107–184. doi:10.1080/713610925

Trust Hammer, B., Fogel, M.L., Hoering, T.C., 1998. Stable carbon isotope ratios of fatty acids in seagrass and redhead ducks. Chemical Geology 152, 29–41. doi:10.1016/S0009-2541(98)00094-1

Vogedes, D., Varpe, O., Søreide, J.E., Graeve, M., Berge, J., Falk-Petersen, S., 2010. Lipid sac area as a proxy for individual lipid content of arctic calanoid copepods. Journal of plankton research 32, 1471–1477. doi:10.1093/plankt/fbq068

Von Biela, V.R., Zimmerman, C.E., Cohn, B.R., Welker, J.M., 2012. Terrestrial and marine trophic pathways support young-of-year growth in a nearshore Arctic fish. Polar Biology 36, 137–146. doi:10.1007/s00300-012-1244-x

Wang, S., Budge, S., Gradinger, R., Iken, K., Wooller, M., 2014a. Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering Sea: tools for estimating sea ice algal contribution to Arctic food web production. Oecologia 174, 699–712. doi:10.1007/s00442-013-2832-3

Wang, S.W., Hollmén, T.E., Iverson, S.J., 2014b. Egg yolk fatty acids as a proxy to quantify diets of female Spectacled Eiders (*Somateria fischeri*). Canadian Journal of Zoology 92, 453–461. doi:10.1139/cjz-2013-0293

Watt, C.A., Ferguson, S.H., 2015. Fatty acids and stable isotopes (δ 13 C and δ 15 N) reveal temporal changes in narwhal (*Monodon monoceros*) diet linked to migration patterns. Marine Mammal Science 31, 21–44. doi:10.1111/mms.12131

Wilce, R.T., Dunton, K.H., 2014. The Boulder Patch (North Alaska , Beaufort Sea) and its Benthic Algal Flora 67, 43–56.

Wold, A., Darnis, G., Søreide, J.E., Leu, E., Philippe, B., Fortier, L., Poulin, M., Kattner, G., Graeve, M., Falk-Petersen, S., 2011. Life strategy and diet of *Calanus glacialis* during the winter–spring transition in Amundsen Gulf, south-eastern Beaufort Sea. Polar Biology 34, 1929–1946. doi:10.1007/s00300-011-1062-6

Zeng, D., Mai, K., Ai, Q., Milley, J.E., Lall, S.P., 2010. Lipid and fatty acid compositions of cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*) and halibut (*Hippoglossus hippoglossus*). Journal of Ocean University of China 9, 381–388. doi:10.1007/s11802-010-1763-4

Vita

Stephanie Smith was born in Houston, Texas. As a child, she attended Sea Camp at Texas A&M University at Galveston (TAMUG) for three summers. After graduating from Tomball High School, she went on to receive her B.S. in Marine Biology at TAMUG. Although she was hoping to explore new states, the Texas coast called her back, and she entered the marine science master's program at the University of Texas Marine Science Institute in August 2012.

Permanent email: sd.smith@utexas.edu

This thesis was typed by the author.